

The Journal of Parasitology

Volume 28

DECEMBER, 1942

Number 6

THE EFFICIENCY OF THE BAERMANN APPARATUS IN THE RECOVERY OF LARVAE OF *HAEMONCHUS CONTORTUS*

A. G. DINABURG

Zoological Division, U. S. Bureau of Animal Industry

The apparatus commonly used for the isolation of nematode larvae from soil was first described by Baermann in 1917. It consisted of a glass funnel closed at the bottom by a clamp placed on a piece of rubber tubing attached to the shank, and a sieve of 1 mm mesh wire covered with a piece of coarse cloth. In order to isolate hookworm larvae from fecal cultures or contaminated soil, the funnel was filled nearly to the brim with filtered and sterilized water and the cloth covered screen containing soil or feces with hookworm larvae was placed in the water in the funnel. Samples of varying sizes were withdrawn from the bottom of the funnel at different intervals and examined for larvae. The temperature of the water was not stated. Cort, Ackert, Augustine and Payne (1922) tested the effect of various changes in the operation of the apparatus upon the recovery of hookworm larvae from soil. They found that the water must be at least 10° F warmer than the soil, that a higher percentage of larvae can be isolated from coarse than from finely divided soils, that a slightly higher percentage of larvae can be isolated from moist than from saturated soil, and that while most of the larvae escape from the soil into the water in the first 6 hours, an appreciable number come out later. They also found that under favorable conditions the percentage of larvae recovered was high but variable, the average being about 52 per cent. Stoll (1923) added known numbers of hookworm larvae to 100 gm samples of different types of soil, and used the Baermann apparatus under the conditions standardized by Cort et al (1922) to recover the larvae. He found that about 90 to 95 per cent of the larvae were recovered from "humus," consisting mainly of vegetation in various stages of decay, about 80 per cent from sand and loam, and about 33 to 50 per cent from clay. Cort, Stoll, and Grant (1926) used water routinely at 45° C, after having found that of 1900-3750 mixed *Ancylostoma* and *Necator* larvae placed on 200 gram lots of moist clay-loam soil, and allowed to remain there

Received for publication, December 30, 1941.

about 24 hours before test isolation, an average of 58.1 ± 1.6 per cent were recovered from Baermann units set up with water at $30-50^{\circ}\text{C}$ and allowed to stand over night before being drawn off. This was in mid-summer at Soochow, China, with air temperatures $27-32^{\circ}\text{C}$.

Mönnig (1930) "made critical tests with this method on *Trichostrongylus*, *Haemonchus contortus*, and *Oesophagostomum columbianum* larvae in fecal cultures and in different types of soil and found that it gave excellent results, comparable to those obtained by Cort et al (1922), with hookworm larvae. The best results were obtained with the coarsest soils and a small apparatus, using funnels of 6-inch diameter."

Parnell (1936) added known numbers of horse strongyle larvae previously isolated by the Baermann apparatus to 40 gm of sterilized horse feces suspended in a cheesecloth bag in a funnel of water. A 50-cc sample was then examined for the number of larvae recovered. When the initial number of larvae was 75 to 100, percentages of 12.5, 25, and 45 were recovered from 3 cultures, respectively. When the number of larvae was 700 to 1,000, percentages of 35, 35, and 50, respectively, were recovered, and when the original number was about 10,000 the recoveries were 45, 65, and 90 per cent, respectively.

Kauzal (1940) tested the effect of various modifications of the Baermann technic on its efficiency for the recovery of infective larvae of *Haemonchus contortus* and *Trichostrongylus* spp. Either the former species or both were used, but the results are not given separately, nor are the methods of obtaining the larvae given. He found that more larvae were recovered when water at room temperature (15° to 22°C) was used than when water at 37°C was put into the funnel. The soil plots containing the larvae were supported on wire gauze in funnels of various sizes. With a 6-inch funnel and 50 gm soil, the average recovery in 11 experiments was 33.6 per cent, while 17 per cent was recovered when 100 gm soil in the same size funnel were used. With a 9-inch funnel and 100 gm soil, 43 per cent were recovered. These tests were carried out under identical conditions, with water at room temperature, and an isolation period of 48 hours. However, the numbers of larvae involved are not given. In other experiments, Kauzal found that when the soil was supported by wire gauze the mean recovery was 36.7 per cent; when no gauze was used it was 58.3 per cent. He concludes that the percentage of larvae recovered by this method is low and varies greatly in individual experiments.

PURPOSE OF EXPERIMENTS

The Baermann apparatus was used regularly to recover larvae of *Haemonchus contortus* from stirred samples of soil, sheep feces, and mixtures of both in experiments on the development and survival of the eggs and larvae of this parasite under outdoor conditions. In view of

the extensive use of the apparatus in the above experiments, and the scarcity of available information on the efficiency of the apparatus in the recovery of larvae of *H. contortus*, the following experiments were performed to calibrate the apparatus under conditions¹ similar to those used in the survival experiments.

MATERIALS AND METHODS

The Baermann apparatus consisted of glass funnels provided with rubber tubes, and clamps at the lower end; of straight-sided screens; and of heavy muslin cloths. The funnels were $7\frac{5}{8}$ inches in diameter across the top; the screens, $7\frac{1}{16}$ inches outside diameter and $2\frac{3}{4}$ inches high, and were made of 16-mesh wire screen. The cloths were washed muslin about 35 cm square and weighed about 25 gm. After each experiment the screens and funnels were first washed, and then dipped into boiling water; the cloths were washed, boiled and dried before using in order to kill any remaining larvae.

The procedure used in each experiment was as follows: Six funnels were filled with water at 40° C, to a level below that of the screens. The sterilized cloths were put in each of 6 screens. One hundred grams of steam-sterilized sandy loam was put into each of 2 screens; and in each of 2 other screens, 100 gm ground sheep feces, free of nematode eggs, except for occasional *Strongyloides papillosus* eggs, while in the third pair, neither soil nor feces was placed. The suspension of infective larvae, collected the day of the experiment from cultures of *H. contortus* eggs, was stirred well before each sampling, and 8 or 10 samples of equal volume drawn up with a pipette. Two of the samples were put into separate bottles as controls and 6 samples were pipetted slowly into each of the screens. If sufficient suspension remained, two more control samples were taken. The water level was then brought to just below the surface of the soil or feces, or above that of the cloth, and the ends of the cloth tucked in. Under these conditions, the funnels contained about 1600 cc of water. After the larvae had been in the apparatus for 48 hours a sample of 50 to 60 cc was drawn from the bottom of each funnel by pinching the rubber tube above the clamp with metal tongs, while loosening the clamp, then relaxing the pressure to flush the whole tube.

The number of larvae in each sample and those in the control bottles were determined by examination either of the whole sample or of a diluted part of it under a dissecting microscope. In most experiments 4 control samples were available; at least 4, and in most cases 5, dilution counts were made on each control, and on each Baermann sample containing large numbers of larvae.

¹ The specifications of the components of the apparatus, initial temperature of the water, length of isolation period, and weight of the soil sample used in these experiments were suggested by Dr. D. A. Shorb of this laboratory.

When large numbers of larvae were available, several experiments with different numbers of larvae were set up at the same time.

RESULTS OF EXPERIMENTS

The results of 27 experiments are graphed in Fig. 1. Because of the wide range of numbers involved a logarithmic scale was used for the numbers of larvae put into the apparatus. The percentage recovered

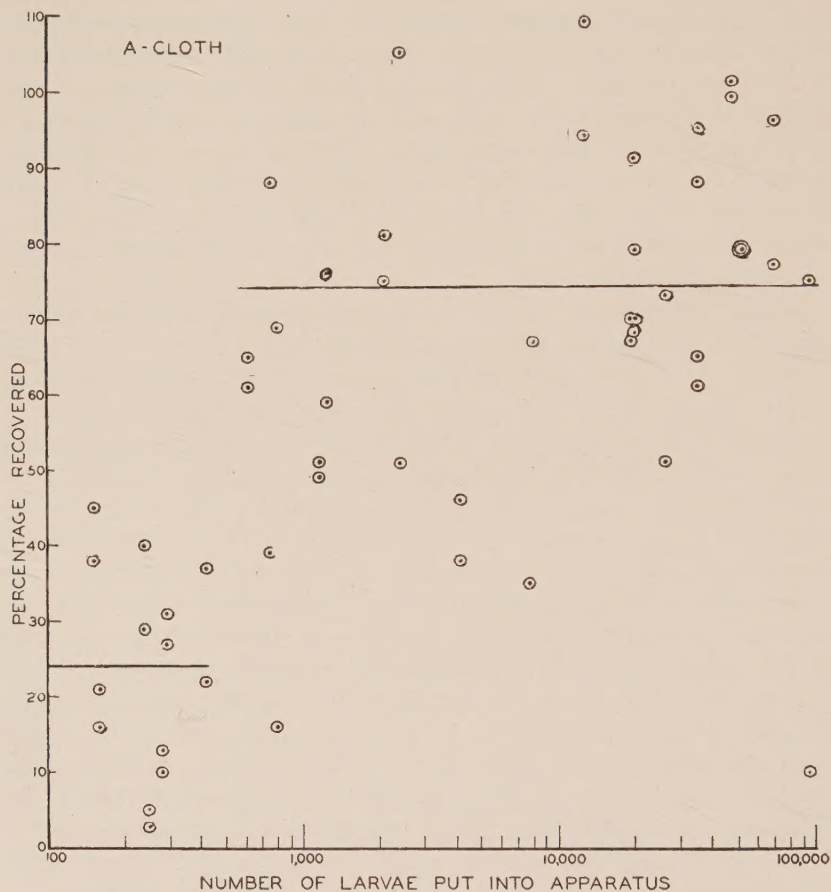


Fig. 1 A. Percentage of infective larvae of *H. contortus* recovered after passage through cloth in the Baermann apparatus.

was obtained by dividing the number recovered from each unit by the average of the numbers in the control samples. In one experiment with 12,600 larvae, there were enough larvae for only one soil unit, from which 60 per cent were recovered. Because of the extensive comparisons involving duplicates, the data for this single unit was omitted.

The data in Fig. 1 indicate that with an input of less than 600 larvae, the percentages recovered after passage through cloth and through soil

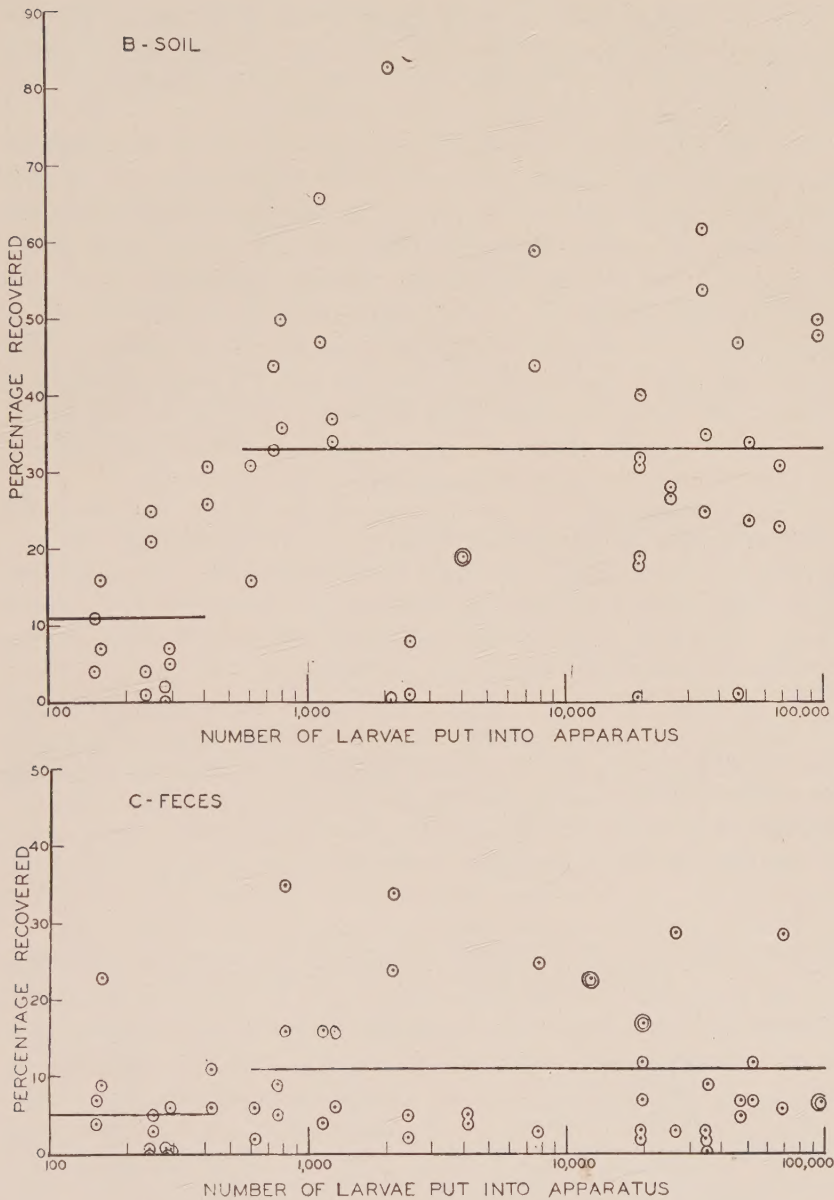


Fig. 1 B, C. Percentage of infective larvae of *H. contortus* recovered after passage through cloth plus 100 gm of soil (B) and cloth plus 100 gm of ground sheep feces (C) in the Baermann apparatus.

are not in line with those recovered from higher inputs. For this reason, the results of 7 experiments with inputs below 600 larvae were treated separately from the results of 20 involving more than 600 larvae.

In the 7 experiments involving inputs of 150 to 420 larvae, the percentage recovered through cloth, soil, and feces averaged 24, 11, and 5, and ranged from 3 to 45, 0 to 31, and 0 to 23 per cent, respectively. One soil unit and 2 feces units did not yield any larvae.

In the experiments with inputs ranging from 600 to 95,400 larvae, the averages of the percentages recovered through cloth, soil and feces were 69, 34 and 11 per cent, respectively. Analyses of variance (Snedecor, 1940) on the percentages recovered in each of the three groups showed that the average percentages differed significantly from each other, but that within each of the groups the variance between experiments were not significantly greater than that within experiments. This second test gave a mean square for variation between duplicates that was about $\frac{2}{3}$ that from the variation between experiments in the same group. This would indicate almost as large a variation between duplicates as between experiments, as is also indicated by the data in Fig. 1. However, closer examination of the data showed that 5 pairs of units in the cloth series differed by more than 22 per cent in their percentage recovery, 2 pairs in the soil series, and 1 pair in the feces series. None of these pairs were found in the same experiments, or at one end of the input range. However, 3 pairs in the cloth series occurred with inputs below 2,500 larvae, and 4 with inputs below 8,000 larvae.

If the data from these 8 pairs are set aside, the average percentage recovery is not changed materially, but new information about the operation of the apparatus can be obtained from the data from the remaining 51 pairs. The new averages of the percentage recoveries for cloth, soil, and feces now become 74, 33, and 11 per cent, respectively. An analysis of variance of the percentages in these reduced groups is shown in Table 1.

TABLE 1.—*Analysis of variance of percentages of larvae recovered by the Baermann apparatus, after passage through various materials*

Material	Source of variation	Degrees of freedom	Variance
Cloth	Between experiments	14	572**
	Between duplicates	15	69
Cloth plus soil	Between experiments	16	470**
	Between duplicates	17	60
Cloth plus feces	Between experiments	18	129*
	Between duplicates	19	50

* Significant difference, by F test.

** Highly significant difference, by F test.

This analysis indicates that the percentages recovered in the successive experiments differ significantly more than do duplicates within the same experiment. From this analysis the variance due to these two factors can be isolated. The variances due to differences between duplicates for the cloth, soil, and feces series are 69, 60, and 50, while the variances due to

differences between experiments are 251, 205, and 40, respectively. Interpreted in terms of the operation of the apparatus, these figures indicate that the variations in the experimental technics before and after the larvae have been in the apparatus, are about the same in the three groups of experiments. The differences between the experiments, after the separation of this experimental error, are much larger than this source of variation in the cloth and soil series, and are due to the inconsistent operation of the apparatus from one experiment to the next. No regular variation of percentage recovery with size of input could be found.

To complete the account of the data, the average recoveries of the 8 pairs of units set aside were 55, 33, and 16 per cent for the cloth, soil, and feces series, respectively. In the cloth series, the variance between experiments was less than that within experiments. No explanation for the results in this group of 8 pairs can be given.

DISCUSSION

The data indicate that the variability in the performance of the apparatus is much more responsible than the variability in the other experimental technics for the differences between experiments with either cloth or soil when more than 600 larvae were put into the apparatus. In experiments with layers of feces these two factors are about equal, perhaps because of the low upper limit on the percentages recovered set by the bulk of material. The reasons for the variability in the performance of the apparatus were not investigated, since the present experiments were not designed for that purpose. However, it has been possible to isolate the effect of the apparatus from the effect of the other technics involved in using the apparatus, and to measure the average percentage recovery of the larvae of *H. contortus* through various materials.

These results bear directly on the interpretation of recoveries of infective larvae of *H. contortus* with the Baermann apparatus, under conditions similar to those of the present experiments. Considering the low average recoveries through 100 gms of soil or feces with inputs of 150 to 420 larvae, and with occasional failure to recover any larvae, it would appear advisable to weigh carefully low or negative recoveries with the apparatus when the initial number in the sample is expected to be low. Even when the initial number is expected to be much larger, the effect of the type of substrate and of the variability of the apparatus upon the net efficiency of the apparatus should be considered in dealing with the numbers of larvae recovered.

SUMMARY

1. The percentage of infective larvae of *H. contortus* recovered after passage in the Baermann apparatus through cloth, cloth plus 100 grams

of sandy loam, and cloth plus 100 grams of ground sheep feces, was determined in 27 experiments.

2. The efficiency of the Baermann apparatus varied with the type of material used as substrate and the initial number of larvae. In 7 experiments involving 150 to 420 larvae, an average of 24, 11, and 5 per cent were recovered after passage through layers of cloth, soil, and feces, respectively, whereas in 15 to 19 experiments with 600 to 95,400 larvae, these averages were 74, 33, and 11 per cent, respectively.

3. In experiments with more than 600 larvae, the variations in percentage recovery between experiments with cloth or soil layers were due mainly to inconsistent operation of the apparatus, rather than to variations in the technic of handling and counting of the larvae before and after passage through the apparatus. The variation in technic is about the same in all three groups.

BIBLIOGRAPHY

- BAERMANN, G. 1917 Eine einfache Methode zur Auffindung von Ankylostomum (Nematoden) Larven in Erdproben. *Geneesk. Tijdschr. Nederl.-Indie* 57: 131-137.
- CORT, W. W., ACKERT, J. E., AUGUSTINE, D. L. AND PAYNE, F. K. 1922 Investigations on the control of hookworm disease. II. The description of an apparatus for isolating infective hookworm larvae from soil. *Am. J. Hyg.* 2: 1-16.
- CORT, W. W., STOLL, N. R. AND GRANT, J. B. 1926 Researches on Hookworm in China. I. Problems and methods of attack. *Am. J. Hyg. Monogr. Ser.* 7: 1-32.
- KAUZAL, G. P. 1940 Experiments on the recovery of sheep nematode larvae from pastures. *J. Council Scient. and Indust. Research Australia* 13: 95-106.
- MÖNNIG, H. O. 1930 Studies on the bionomics of the free-living stages of *Trichostrongylus* spp. and other parasitic nematodes. *Rep. Director Vet. Serv., Dept. Agric., Union South Africa* 16: 175-198.
- PARNELL, I. W. 1936 Studies on the bionomics and control of the bursate nematodes of horses and sheep. II. Technique. *Canad. J. Research* 14(D): 71-81.
- SNEDECOR, G. W. 1940 *Statistical Methods*. Collegiate Press; Ames, Iowa. 3 ed.
- STOLL, NORMAN R. 1923 Investigations on the control of hookworm disease. XXIV. Hookworm cultures with humus, sand, loam and clay. *Am. J. Hyg.* 3 (July Suppl.): 1-36.

THE EFFECT OF SULFONAMIDE COMPOUNDS ON THE GROWTH OF *ENDAMOEBIA HISTOLYTICA* IN CULTURE*

ENID C. RODANICHE AND JOSEPH B. KIRSNER

Department of Medicine, University of Chicago

The sulfonamide compounds have received in general less attention in the therapy of parasitic than of bacterial diseases. A review of the literature reveals only one reference to the use of any of these compounds in amebic infection (Saini, 1940). Since *Endamoeba histolytica* is readily maintained in culture, it was decided to use the culture method to study the action of the sulfonamide compounds on this organism. Sulfanilamide, sulfapyridine, sulfathiazole and sulfaguanidine were given extensive trial. Several experiments were conducted also with sulfadiazine as noted in the text.

Strains of Endamoeba histolytica. The majority of the experiments were made with a strain of *E. histolytica* which had been isolated from the stool of a patient with amebic dysentery 2½ years previously and had been maintained in culture since that time. A few experiments were performed with strains freshly isolated from recent cases and carriers.

Media. For cultivation of *E. histolytica* a slight variation of the Cleveland and Collier (1930) medium was utilized, the variation consisting in the use of one part of sheep serum and 7 parts of Locke's solution without dextrose in place of the usual serum and physiological salt solution to make up the fluid portion of the medium. Very heavy growth has been obtained consistently in this medium with stock strains. Growth with old strains usually reaches a maximum in 3 days with subsequent decline and death in 5 to 9 days. With freshly isolated strains this process is usually accelerated.

As is well known *E. histolytica* cannot be maintained in culture in the absence of bacteria. In fact, the type and quantity of the accompanying bacteria or fungi profoundly affects the success or failure of cultivation. Faust (1941) has suggested that some type of symbiotic relationship is involved. Our stock culture has become stabilized and uniformly shows growth of coliform bacilli, alpha and gamma streptococci and staphylococci. More recently isolated strains present a wider variation in the bacterial flora. Such a relationship of course complicates the evaluation of the effect of the drugs, but may present a truer picture of the probable action of these compounds in the body.

Received for publication, January 21, 1942.

* This work was done under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

Sulfonamide Compounds. Saturated solutions of the sulfonamide compounds were prepared by adding an excess of these substances to the Locke's solution-serum mixture. The mixture was then placed in the incubator at 37.5° C for 2 hours and the excess drugs removed by filtration through a Berkefeld N filter; this process also served to sterilize the filtrate. Exactly 5 cc of the fluid was then used to overlay the liver infusion agar slants.

For some experiments a 3 mm loopful of the respective crystalline compounds was added to the saturated drug slants. No effort was made to sterilize the drugs before addition. However, it was not found that the contamination so introduced produced any significant alteration in the cultures.

The initial experiments were designed to give a general idea of the action, if any, of the sulfonamide compounds on the amebae. For this purpose, four tubes containing respectively sulfanilamide, sulfathiazole, sulfaguanidine, and sulfapyridine in solution were inoculated and incubated at 37.5° C. A control tube without drugs was similarly treated. The cultures were examined periodically to determine the presence or absence and the relative abundance of the amebae by transferring a small amount of the sediment to a glass slide and examining under the microscope. The results are shown in Table 1. The amebae were found to

TABLE 1.—*Effect of the addition of sulfonamide compounds in saturated solution on the life of Endamoeba histolytica in culture*

Compound added in saturated solution	Life of culture in days							
	1	3	5	7	10	12	15	20
Sulfanilamide	P	P	0	0	0			
Sulfaguanidine	P	P	P	P	0	0		
Sulfathiazole	P	P	P	P	P	P	0	0
Sulfapyridine	P	P	P	P	0	0		
Control	P	P	P	0	0			

P indicates that living forms were present.

0 indicates that no living forms were observed.

survive in the saturated sulfanilamide preparation for 3–5 days, in sulfaguanidine for 7–10 days, in sulfathiazole for 12–15 days, in sulfapyridine for 7–10 days, and in the control for 5–7 days. In general growth was less abundant in the cultures containing the drugs than in the control and was particularly scanty in the tube containing sulfanilamide. This preliminary work established the fact that *E. histolytica* was able to survive in cultures containing saturated solutions of three sulfonamide compounds as long or longer than in controls. The growth of this organism, however, was diminished.

The question then arose as to whether the survival of *E. histolytica* was due to a gradual diminution in the activity of the sulfonamide drugs with the passage of time. To study this factor culture tubes prepared as

previously described but containing a loopful of the crystalline compounds were employed. Since the crystals could be readily identified by examination of the sediment, one could be certain that the drugs remained in excess throughout the observation period.

The results of this experiment appear in Table 2. It will be noted

TABLE 2.—*Effect of the addition of sulfonamide compounds in the crystalline state on the persistence of Endamoeba histolytica in culture*

Compound added in crystalline form	Life of culture in days									
	1	3	5	7	10	12	15	20	24	26
Sulfanilamide	0	0	0							
Sulfaguanidine	P	P	P	P	P	P	0	0		
Sulfathiazole	P	P	P	P	P	P	P	P	P	0
Sulfapyridine	P	P	P	P	P	P	0			
Control	P	P	P	0	0					

P indicates that living forms were present.
0 indicates that no living forms were observed.

that, with the exception of sulfanilamide, growth persisted in the presence of the crystalline drugs for periods longer than were noted with the saturated solutions and for a much longer time than was observed in the control. In the culture containing crystals of sulfanilamide, however, growth had ceased within 24 hours. The amebae showed neither positive nor negative chemotaxis toward the sulfonamide crystals, progressing towards, among and away from them freely. It was noted again, however, that growth in the cultures containing the drugs was less abundant than in the control.

Having established by this preliminary work that amebae will survive, though in diminished numbers, in saturated and supersaturated solutions of sulfapyridine, sulfathiazole and sulfaguanidine and in saturated solutions of sulfanilamide, our next step was to apply a more accurate quantitative method to determine the actual degree of inhibition. For this purpose, the test and control cultures were inoculated with exactly 0.1 cc of an evenly dispersed suspension of *E. histolytica*. At various periods the fluid contents of the tubes were well stirred, and 0.05 cc quantities were removed to slides, covered with cover-glasses, and the total number of amebae in 16 fields taken along the diagonals at regular intervals was counted under the low power of the microscope. The average number of organisms per field was derived from this procedure. The entire preparation was first examined rapidly to make certain there was even dispersion and no clumping. Careful check indicated that this method gave a sufficiently accurate picture of the relative abundance of the organisms. Examinations were made at 1, 2, 3, 5, 6, 7, 10, 17, 24 and 27-day intervals. Growth was so scanty after an interval of 17 or more days that counts were not made. The whole preparation was examined, however, and if any viable organisms were

detected, the culture was considered positive. These results appear in Table 3. Reference to this table establishes two major findings:

TABLE 3.—*Effect of saturated solutions of sulfonamide compounds on the growth of Endamoeba histolytica and accompanying bacteria in culture*

Compound added in saturated solution		Average number of amebae and bacteria in culture after varying periods of incubation*											
		Days											
		1	2	3	5	6	7	10	17	24	27		
Sulfanilamide	Amebae	0.25	0.1	0	0								
	Bacteria	680M	380M	250M			90M						
Sulfaguanidine	Amebae	4.1	30.7	37.0	16.0	10.0	9.0	0	0				
	Bacteria	920M	370M	400M			57M						
Sulfapyridine	Amebae	6.0	18.9	49.2	13.5	11.9	9.9	0	0				
	Bacteria	1B	640M	590M			170M						
Sulfathiazole	Amebae	7.9	11.5	16.9	5.5	3.2	1.6	4.0	P	P	0		
	Bacteria	950M	520M	410M			90M						
Control	Amebae	17.9	63.4	64.4	0	0							
	Bacteria	1B	930M	760M			150M						

* Number of amebae equals the average count per 16 fields.

Number of bacteria equals the number per cc of culture fluid.

B indicates billion.

M indicates million.

P indicates living amebae were present.

0 indicates no living amebae were found.

1. All the sulfonamide compounds tested produced inhibition of the growth of *E. histolytica* in culture. This inhibition was most marked in the case of sulfanilamide, followed in order by sulfathiazole, sulfaguanidine and sulfapyridine.

2. Viable amebae persisted in culture for much longer periods of time when the drugs other than sulfanilamide were present than in the control preparations. Living amebae disappeared from the cultures which were not drug-treated between the 3rd and 6th day, whereas they persisted in the cultures containing sulfaguanidine, sulfapyridine and sulfathiazole from 9 to 24 days. The fact that viability was maintained for longer periods in this series than in the preliminary experiments is attributable to the fact that these cultures were submitted to thorough agitation at each examination with consequent dispersion of local accumulations of toxic waste products, whereas previously the sediment alone was examined after very little agitation of the supernatant fluid.

It is of interest to note that maximum growth occurred on the third day for all the cultures except that containing sulfanilamide, and was then followed by rapid decline in the control and by gradual decline in the test preparations.

There was no marked morphological change in the drug-treated amebae, except that there was usually prolonged persistence of large forms. Such large forms ordinarily appear in the untreated cultures only during the early growth phase and then are supplanted by smaller forms during the period of maximum growth and decline. This observation

might indicate a prolongation of the growth phase under the action of the sulfonamides. Amebae from both untreated and drug-treated cultures showed the same degree of motility and contained approximately the same amount of ingested starch granules and bacteria. Stained smears of both control and drug-treated amebae showed typical morphology with nuclear divisions appearing in stained individuals in both groups. However, no attempt was made to determine the relative number of fissions occurring among the individuals of the control and test groups. Encystation occurred in the drug-treated cultures as frequently as in the controls, and the cysts appeared typical. It may be stated that encystation is less frequent and more irregular in strains maintained for several passages on artificial media than in freshly isolated strains. No stimulation of encystment, however, was provided by the sulfonamide compounds.

Since, as previously stated, the growth of amebae in culture involves a probable symbiotic relationship with bacteria, the question arises as to what effect the sulfonamides exert on the bacterial flora of the cultures, and to what extent this effect is reflected in the ameba population. To study this problem estimates were made of the relative numbers and types of bacteria present in the various culture tubes. One-tenth cc of the well agitated culture fluid employed for the ameba counts was withdrawn and used to prepare serial dilutions up to 1-10 million in distilled water. These dilutions were then utilized to seed veal infusion agar pour plates which were incubated at 37.5° C for 48 hours. At this time the number of colonies developing per plate was counted and the number of viable organisms per cc of culture fluid thus estimated. Identification of typical colonies was made also by staining, fermentation and other tests. The high dilutions employed were believed to nullify any inhibitory effect of the drugs on the growth of viable organisms in the plates. These results appear in Table 3, and show that the sulfonamides tested exerted only a slight inhibitory effect on the growth of the bacteria. Furthermore, this action did not appear to be selective, for both gram positive cocci and gram negative bacilli persisted. The inhibition of *E. histolytica* in culture apparently, then, cannot be correlated with a simultaneous bacteriostasis.

The above experiment was repeated using culture fluids containing a loopful of the crystalline drug. The method was as previously described except that observations were not made beyond the 17-day period. The results are shown in Table 4. As in the preliminary experiments, amebae disappeared in 24 hours in the presence of crystalline sulfanilamide. Distinct inhibition of growth was produced by the other compounds, although the order of the inhibitory effect was changed, the alteration being most pronounced in the case of sulfaguanidine.

The degree of stasis of amebic growth may coincide to some extent with the solubility of the drugs used: sulfanilamide, which exerted the

TABLE 4.—*Effect of supersaturated solutions of the sulfonamide compounds on the growth of Endamoeba histolytica in culture*

Compound added in crystalline form	Average number of amebae counted in 16 fields after periodic intervals of incubation						
	Days						
	1	2	3	6	7	8	17
Sulfanilamide	0	0	0				
Sulfaguanidine	0.94	2.7	8.2	16.4	20.1	19.6	P
Sulfapyridine	1.9	28.1	12.6	6.0	5.3	3.3	0
Sulfathiazole	0.7	20.1	15.4	6.2	3.8	1.7	P
Control	0.9	40.6	66.6	3.1	2.5	2.1	0
Saturated solution of sulfanilamide ..	1.0		0.2	0		0	
Above solution diluted 1 to 5	4.1		8.0	12.2		1.9	

P indicates living amebae were present.

0 indicates no living amebae were found.

greatest inhibitory effect, is also the most soluble. To further elucidate this question, a saturated solution of sulfanilamide at 37.5° C was diluted 1–5 times with normal serum-Locke's solution and then inoculated with *E. histolytica*. A quantitative estimation of the degree of growth was made over a period of 8 days and the result compared with that observed in a control tube and in a tube containing a saturated solution of sulfanilamide. The results appear in the lower part of Table 4. It will be readily noted that the inhibitory effect of sulfanilamide greatly decreased by dilution of the drug, but that even in this dilution growth was by no means as great as that in the control preparation.

Sulfadiazine. A few tests of the effect of sulfadiazine were made. It was found that *E. histolytica* grew well in culture media containing this compound in saturated and in supersaturated solution. Inhibition was most noticeable in the first two days of culture. On the second day an average count of 2.4 amebae in 16 fields was obtained in the sulfadiazine-saturated preparation, whereas the average count in the control was 15.4. However, growth rapidly increased in the drug-treated culture, and living amebae were still fairly numerous after 11 days of incubation, although life had ceased in the control culture at the end of 7 days. It was very interesting to note that *E. histolytica* readily ingested the smaller crystals of sulfadiazine, a phenomenon not observed with the other sulfonamide compounds, although it may have occurred occasionally. The presence of these ingested crystals did not seem to impair the activity of the amebae during the 5 or 10 minute periods of observation.

To clarify further the action of sulfanilamide on the growth of *E. histolytica*, the following experiment was conducted. Four of the regular culture tubes were inoculated with 0.1 cc of an evenly dispersed suspension of amebae and the tubes were incubated for 24 hours. At the end of this time, the fluid contents were well mixed and the average number of amebae in 16 fields of a slide preparation counted as described previously. The tubes were then centrifuged, the supernatant was discarded and re-

placed as follows: to tube No. 1 was added exactly 5 cc of untreated serum-Locke's solution; to tube No. 2, 5 cc of serum-Locke's solution saturated with sulfanilamide; to tube No. 3, the same plus a loopful of crystalline sulfanilamide; and to tube No. 4, serum-Locke's solution with added sulfanilamide and para-amino-benzoic acid. The tubes were then replaced in the incubator, and microscopic examination with counts of 16 fields performed at the end of 15 minutes, 2½, 5, 24 and 48 hours. Examination without count was also made in 96 hours. These results are recorded in Table 5.

TABLE 5.—*Effect of sulfanilamide on the growth of amebae in culture*

Culture tube No.	Av. no. of amebae after 48 hrs. incubation	Further treatment	Av. no. of amebae after incubation for hours						
			¼	2½	5	24	48	96	
1	25.1	Supernatant replaced with untreated serum-Locke's solution	24.7	31.5	66.6	84.2	20.0	P	
2	21.4	Supernatant replaced with serum-Locke's solution saturated with sulfanilamide	20.8	15.1	9.5	21.6	13.9	P	
3	17.8	Supernatant replaced with serum-Locke's sol. and crystals of sulfanilamide added	15.8	16.3	15.6	6.9	2.4	0	
4	16.3	Supernatant replaced with serum-Locke's sol. containing crystals of sulfanilamide and p-amino benzoic acid	11.2	3.3	4.4	4.7	2.7	P	

P indicates living amebae were present.
0 indicates no living amebae were found.

It will be noted that the number of amebae remained stationary in all tubes for 15 minutes, except in tube No. 4 in which there was a significant decline in the ameba count. After an interval of 15 minutes the number increased up to the 24-hour period in tube No. 1, declined up to the 5-hour period in tube No. 2 and then rose, remained stationary up to the 5-hour period in tube No. 3 and then declined, and declined steadily in tube No. 4. A "latent period" apparently operates in the inhibition of amebic growth by sulfanilamide as well as in bacteriostasis. It may be noted that life persisted in the preparation containing crystalline sulfanilamide for 2-4 days whereas in previous experiments it had disappeared in 24 hours. This may be attributed to a difference in the preparation of the tubes with a much higher initial inoculum, and also to frequent agitation of the fluid with dispersion of particles. It is of interest that although the addition of p-amino-benzoic acid seemed to precipitate a more rapid decrease in the number of amebae it permitted the persistence of living amebae beyond the period tolerated in the preparation containing crystalline sulfanilamide alone.

Adaptation to Sulfanilamide. An attempt was made to increase the

sulfanilamide tolerance of the amebae by continuous passage in culture media containing this compound. Such an adaptation was readily effected, growth appearing more heavy with each of 4 successive passages. For the fifth passage, the adapted amebae were inoculated into a tube containing crystals of sulfanilamide. The organisms were able to tolerate this treatment for more than 3 days, although growth remained scanty.

Recently Isolated Strains. Two strains freshly isolated from 2 different patients with amebic dysentery were inoculated on the second passage in artificial media into culture tubes containing saturated solutions of sulfanilamide, sulfaguanidine, sulfathiazole and sulfapyridine. Results were in general comparable to those obtained with the old stock strain.

In addition control culture tubes and tubes treated with sulfaguanidine, sulfapyridine and sulfathiazole were seeded with fecal material containing cysts only of *E. histolytica* obtained from a carrier. Excystation and growth occurred in the control preparation and the preparation treated with sulfathiazole in 24 hours. However, in the cultures containing sulfaguanidine and sulfapyridine excystation was delayed for 48 hours. Growth persisted for 1 to 2 days in the drug-treated cultures beyond that in the controls.

Black and his co-workers (1941) have demonstrated that liver extract is more effective than p-amino-benzoic acid in counteracting the action of sulfaguanidine in vivo. Since the culture medium used in the present experiments contained liver infusion agar, the possibility arose that the liver might have diminished the inhibitory effect of the drugs. An experiment was therefore devised, using as a culture medium only serum-Locke's solution, a preparation suitable for the cultivation of amebae as shown originally by Craig (1934). In our experience growth is much scantier and more irregular in this medium than in the Cleveland and Collier medium, in view of which its use has been avoided heretofore. Culture tubes of this fluid containing the five sulfonamide compounds previously described in saturated solution were inoculated with *E. histolytica*, incubated and examined at intervals. Growth occurred in all tubes and no greater inhibition was apparent than when cultures containing liver infusion agar were employed.

DISCUSSION

Although the results herein reported do not appear very encouraging, we feel that the sulfonamide compounds merit clinical trial in amebiasis and amebic dysentery. Such a trial becomes more important when one recalls the lack of correlation between the in vivo and in vitro activity of the sulfonamides in other conditions. Sulfanilamide appears to be the drug of choice, treatment perhaps being supplemented to advantage with retention enemas in which a high concentration of the compound may be employed. It may be noted that Saini obtained successful therapy with

sulfamethylthiazole in amebic dysentery in a child. The marked inhibition of *E. histolytica* in culture by the sulfonamide compounds, moreover, seems to indicate that synthesis of further compounds might prove of value.

Even though clinical trial may be unsuccessful, however, the use of *E. histolytica* seems to offer particular promise in the study of the action of the sulfonamides. This protozoon has the advantage that it is large, has definite structure and can be studied in the stained and living state.

SUMMARY

1. Five sulfonamide compounds, sulfanilamide, sulfathiazole, sulfapyridine, sulfaguanidine and sulfadiazine, all showed inhibitory action on the growth of *Endamoeba histolytica* in culture. Of these the action of sulfanilamide was the most pronounced.

2. At the same time these compounds often permitted persistence of living amebae in culture for longer periods of time than were obtained with media containing no drugs.

3. *Endamoeba histolytica* readily developed tolerance to the action of sulfanilamide.

BIBLIOGRAPHY

- BLACK, S., McKIBBIN, J. M. AND ELVEHJEM, C. A. 1941 Use of sulfaguanidine in nutrition experiments. *Proc. Soc. Exper. Biol. and Med.* **47**: 308-310.
- CLEVELAND, L. R. AND COLLIER, JANE 1930 Various improvements in the cultivation of *Entamoeba histolytica*. *Am. J. Hyg.* **12**: 606-613.
- CRAIG, C. F. 1934 Amebiasis and Amebic Dysentery. Charles C. Thomas, Springfield, Ill.
- FAUST, E. C. 1941 Recent laboratory work throwing light on clinical amebiasis. *Rev. Gastroenterol.* **8**: 197-203.
- SAINI, M. 1940 I derivati metiltiazolici nell cura dell' amebiasi. *Rinasc. Med.* **17**: 679.

NOTES ON NEMATODES FROM THE LUNGS AND FRONTAL SINUSES OF NEW YORK FUR-BEARERS

FRANS C. GOBLE AND ARTHUR H. COOK

Wildlife Research Center, N. Y. State Conservation Dept., Delmar, New York

Since the time of Redi scattered reports on the occurrence of various lung and sinus nematodes in fur animals have appeared, but strikingly little has been recorded which would indicate to what extent these infections are present in the wild. This article summarizes the parasitological findings in the respiratory tracts of the fur-bearers examined by us during the three years ending January 15, 1942.

Previous work on this group of hosts in this area has not been extensive. Hanson (1932) recorded lung and sinus worms from minks in northern New York and Stegeman (1939) noted skull lesions attributable to parasitism in skunks from the central part of the state. Goble and Cook (1941) observed the occurrence of lungworms in foxes in the Helderberg region and examinations of skunks in the vicinity of Albany led to the identification of the sinus worms and to the discovery of a new species of lungworm (Goble, 1942 a and b).

MATERIAL

All of the hosts examined were wild-trapped in New York State. The great majority of the specimens were taken in three counties bordering on the Hudson river within a radius of 50 miles of Albany. The remainder of the animals came from various other places in the state. The hosts examined are listed in Table 1 and the areas which were extensively collected may be seen in Map 1. The three counties in which more than two parasites were found were the ones in which the largest collections were made.

INCIDENCE

Table 1 indicates in a general way the extent of occurrence of respiratory parasites in the hosts examined. The percentages are presented only in order to convey the incidences in simple terms.

Data concerning relative infection with *Capillaria aerophila* in the two sexes was furnished by examination of a large series of red foxes from a limited region in southwestern Albany county and northern Greene county. In this area (the Helderberg plateau) a total of 68 males and 54 females was taken during the months of November and December in the years 1940 and 1941. The incidence of *C. aerophila* in males was 45.6% and in females 48.1%. It seems permissible, therefore, to com-

bine the records for the two sexes in determining the incidence of this parasite. Incidences in three areas within this region ranged from 41 to 52% but the differences were not significant. *C. aerophila* occurred in 22.5% of the red foxes in Washington county but was not found in any of 25 animals from central New York.

The incidence of *Crenosoma vulpis* was significantly greater in males than in females (28% and 15.7%). Analysis of these data above gave

TABLE 1.—Occurrence of lung and sinus worms in fur-bearers in New York

Host	Number examined		Adults infected		Parasite
	Juvenile	Adult	Number	Per cent	
Opossum (<i>Didelphys virginiana</i>) .	8	14	0*		
Red fox (<i>Vulpes fulva</i>)	6†	184	39 66	21 36	<i>Crenosoma vulpis</i> (Dujardin, 1845) <i>Capillaria aerophila</i> (Creplin, 1839)
Gray fox (<i>Urocyon c. cinereoargenteus</i>)	1	88	15 6	17 7	<i>Crenosoma vulpis</i> <i>Capillaria aerophila</i>
Mink (<i>Mustela v. vison</i>)	0	44	22 4*	50 40	<i>Filaroides bronchialis</i> (Gmelin, 1790) <i>Skrjabinogylus nasicola</i> (Leuckart, 1842)
New York weasel (<i>Mustela frenata noveboracensis</i>)	15	15	6 4*	40 40	<i>Filaroides bronchialis</i> <i>Skrjabinogylus nasicola</i>
Bonaparte weasel (<i>Mustela c. cicognani</i>) .	0	5	0*		
Skunk (<i>Mephitis nigra</i>)	6	57	11 18*	19 72	<i>Crenosoma zederi</i> Goble, 1942 <i>Skrjabinogylus chitwoodorum</i> Hill, 1939
Raccoon (<i>Procyon l. lotor</i>)	6	20	2	10	<i>Crenosoma</i> sp. (possibly <i>C. striatum</i> or <i>C. taiga</i>)
Beaver (<i>Castor canadensis</i>) . . .	5	4	0		
Muskrat (<i>Ondatra z. zibethica</i>) .	18	180	0		

* The frontal sinuses were examined in the following numbers of adults: 14 opossums, 10 minks, 10 N. Y. weasels, 5 Bonaparte weasels and 25 skunks. All juveniles of these species were examined for sinus worms and found negative.

† Animals designated as juveniles were all collected during the spring and summer and with the exception of one red fox, which was infected with *Crenosoma vulpis*, no lungworms were encountered in any of the young specimens.

a χ^2 value of 5.43 which is significant with one degree of freedom. We are unable to offer any explanation for this difference at the present time. The incidence of *C. vulpis* was the same in red and in gray foxes and did not differ between the four counties where the largest fox collections were made.

PATHOLOGY

Due to the methods employed in killing trapped animals it is difficult to interpret accurately the significance of gross lesions in the lungs and the evaluation of the tissue changes attributable to the worms depends on

microscopic study of sectioned material. Two cases, however, which were uncomplicated by mechanical injury, are reported here.

In September, 1941, we received an immature female red fox, trapped at Piseco Lake, which was cachectic, poor in pelage and weak. Post-mortem examination revealed bilateral confluent bronchopneumonia and over 100 worms (*Crenosoma vulpis*) were exposed by cutting down the main bronchi. We estimate the total number of adult worms in the lung to have been over 300. Hanson (1933) reported 55 as the maximum



MAP 1. Known distribution of lung and sinus worms in New York fur-bearers. A, *Capillaria aerophila*; B, *Filaroides bronchialis*; C, *Skrjabinigylus chitwoodorum*; N, *Skrjabinigylus nasicola*; S, *Crenosoma* sp.; V, *Crenosoma vulpis*; Z, *Crenosoma sederi*.

infection with this parasite observed in ranch foxes. Another young red fox, trapped within 100 yards of the above on the same day and undoubtedly a litter mate, exhibited the same symptoms and died in captivity two days later but was not available to us for autopsy.

Early in January, 1942, an emaciated adult female skunk was brought to the laboratory. It was found dead near a highway and was suspected of having been killed by an automobile. Autopsy revealed that the cause of death was not traumatic. Examination of the head showed that an

abscess existed in the region of the frontal sinuses and that the bones dorsal and postero-ventral to the sinuses were eroded away so that the cranial cavity was continuous with the abscessed region which was exposed when the skin was removed from the top of the head. The porosity of the bone and the location of the lesions suggested that this condition might be the result of a serious sinus worm infection but no evidence of recent presence of worms could be found.

DISCUSSION

Although our examinations of the lungs and frontal sinuses of opossums disclosed no parasites, we have certain indications that both lung and sinus worms occur in these animals. We have in our collection a micro-slide preparation labelled "opossum lung," which was discarded from the histological collection of the University of Michigan Anatomy Department. This preparation shows nematodes in two bronchi, surrounded by mucous and cellular exudate, with accompanying peribronchitis. The date, locality and species of opossum are unknown. Weidman (1923) reported lung and sinus worms from opossums at the Philadelphia Zoo.

In the light of Hobmaier's (1941b) recent suggestion that foxes might sometimes be infected with the skunk lungworm, *Crenosoma mephitis*, it is interesting to observe that *Crenosoma vulpis* was the only species of that genus which occurred in foxes occupying the same range as the skunks which harbored *Crenosoma zederi*.

Disfigurement of the frontal region of the skull in various mustelids has been reported by a number of observers and previous to the description of *Skrjabinogylus chitwoodorum* Hill, 1939, these lesions were attributed to *S. nasicola* (often erroneously reported as *Filaroides mustelarum*). Recent identifications of sinus worms from five species of skunks (Hill, 1939; Hobmaier, 1941a; Goble, 1942a) seems to indicate that *S. chitwoodorum* is typical in the MEPHITINAE and that *S. nasicola* is the form commonly found in the MUSTELINAE (Hanson, 1932; Swales, 1938).

Swales found *Skrjabinogylus nasicola* in *Mustela cicognani anguinae* and his records of skull lesions point to its presence in *M. arctica*, *frenata* and *longicauda* and to its absence in *M. vison*. We believe, however, the presence or absence of frontal dilatations is not a good index of the incidence of these parasites. We examined the sinuses of ten minks and ten weasels and in each species found infections in four of the ten. No external enlargement or abnormality was observed. On the other hand, no worms were found on opening the sinuses of a Bonaparte weasel which had an enlarged frontal region. The conditions under which alterations of the bone take place are obviously not well understood. Our Bonaparte weasels, which were negative, were taken in the same locality that yielded New York weasels with *S. nasicola* and *Filaroides bronchialis*.

Since muskrats belong to a new world genus and their presence in Europe is the consequence of introductions from this continent, the discovery by Schulz, Orlow and Kutass (1933) of the lungworm *Rodentocaulus ondatrae* in this host in the U.S.S.R. poses the question of whether this parasite was originally an inhabitant of muskrats in this hemisphere or whether it is a recent acquisition to the pulmonary fauna of *O. zibethica*. To our knowledge this nematode has not been found in North America.

SUMMARY

Observations on the incidence and identity of the parasites of the lungs and frontal sinuses of fur-bearers in New York State are recorded. These include: *Capillaria aerophila* and *Crenosoma vulpis* in red and gray foxes, *Skrjabinigylus nasicola* and *Filaroides bronchialis* in minks and New York weasels, *Skrjabinigylus chitwoodorum* and *Crenosoma zederi* in skunks and *Crenosoma* sp. in raccoons.

BIBLIOGRAPHY

- GOBLE, F. C. 1942a *Skrjabinigylus chitwoodorum* from the frontal sinuses of *Mephitis nigra* in New York. J. Mamm. **23**: 96-97.
- 1942b *Crenosoma zederi* n. sp. (Nematoda: Metastrongyloidea) a new lungworm from the skunk (*Mephitis nigra*). J. Parasitol. **28**: 381-384.
- GOBLE, F. C. AND COOK, A. H. 1941 Some lungworm records from foxes in New York. J. Mamm. **22**: 456.
- HANSON, K. B. 1932 Parasites of minks and their control. Circular U. S. Dept. Agric. Bi-1235: 1-8.
- 1933 Tests of the efficacy of single treatments with tracheal brushes in the mechanical removal of lungworms from foxes. J. Am. Vet. Med. Assn. **82** (n.s. 35): 12-33.
- HILL, W. C. 1939 The nematode *Skrjabinigylus chitwoodorum* n. sp. from the skunk. J. Parasitol. **25**: 475-478.
- HOBMAIER, M. 1941a Extramammalian phase of *Skrjabinigylus chitwoodorum* (Nematoda). J. Parasitol. **27**: 237-239.
- 1941b Newer aspects of the lungworm (*Crenosoma*) in foxes. Am. J. Vet. Res. **2**: 352-354.
- SCHULZ, R. E., ORLOW, I. W., AND KUTASS, A. J. 1933 Zur Systematik der Subfamilie Synthetocaulinae Skrj., 1932, nebst Beschreibung einiger neuer Gattungen und Arten. (Vorl. Mitt.) Zool. Anz. **102**: 303-310.
- STEGEMAN, L. C. 1939 Some parasites and pathological conditions of the skunk (*Mephitis mephitis nigra*) in central New York. J. Mamm. **20**: 493-496.
- SWALES, W. E. 1938 *Skrjabinigylus nasicola* (Leuckart, 1842) Petrow, 1927, a nematode parasitic in the frontal sinuses of American Mustelidae. Liv. Jub. Prof. Travassos, pp. 455-458.
- WEIDMAN, F. D. 1923 Parasites, in Fox: Disease in Captive Wild Animals and Birds; Philadelphia.

A STRAIN OF THE MOSQUITO *Aedes aegypti* SELECTED
FOR SUSCEPTIBILITY TO THE AVIAN MALARIA
PARASITE *Plasmodium lophurae*

WILLIAM TRAGER

Department of Animal and Plant Pathology, Rockefeller Institute for Medical
Research, Princeton, N. J.

When a group of mosquitoes, of a species known to be susceptible to a particular kind of malaria parasite, is allowed to feed on an infected host showing many gametocytes in its blood, some of the mosquitoes become very heavily infected, while others do not become infected at all. That this susceptibility or immunity is a hereditary character of the individual mosquito was conclusively shown by Huff (1927, 1929, 1931). By selective breeding he could in a few generations increase or decrease greatly the percentage of individuals of *Culex pipiens* susceptible to infection by *Plasmodium cathemerium*, depending upon whether the successive generations were reared from susceptible or immune mothers, respectively. The highly inbred lines of mosquitoes rapidly lost their fertility and a strain with higher susceptibility or immunity than the original stock could not be permanently established.

Experiments somewhat similar to those of Huff, but using *Aedes aegypti* and *Plasmodium lophurae*, have led to the establishment of a large colony of *A. aegypti* which for over a year has consistently proved to be at least twice as susceptible as the stock colony from which it was derived.

In his original description of *P. lophurae* Coggeshall (1938) reported that 9 out of 15 *A. aegypti* which fed on an infected chick showed oöcysts when they were dissected 6 days later. The mosquitoes were obtained from the colony maintained by me at the Rockefeller Institute in Princeton. Later work by Coggeshall (1941a), Huff (1940), and myself with the same strain of *Aedes aegypti* has indicated that the percentage of infection is usually much less than 60%. Huff (1940) found that the parasites did not develop at all in *A. aegypti* at temperatures less than 28° C, a fact which had been independently observed early in the course of the present work. But even if the mosquitoes were kept at 30° C the percentage of infected individuals obtained from groups fed on chicks showing many gametocytes was always low. Much better results were obtained by Laird (1941) with the closely related mosquito *A. albopictus* which proved to be about 90% susceptible to *P. lophurae*. *Culex restuans* and *Aedes atropalpus* also were highly susceptible and showed sporozoites

in the salivary glands (Laird, 1941). Interestingly enough, *P. lophurae* produced fairly heavy stomach infections in *Anopheles quadrimaculatus* (Coggeshall, 1941a), but in only one mosquito have sporozoites thus far been found (Hurlbut and Hewitt, 1941). Of 167 *C. pipiens* dissected, 1.0% showed infection by *P. lophurae* (Coggeshall, 1941a).

METHODS

A strain of *P. lophurae* obtained from Coggeshall and maintained by intracerebral passage in baby chicks (Trager, 1941) was used, except in one experiment in which use was made of a sub-strain maintained in ducks (Wolfson, 1940). Mosquitoes were allowed to feed only on heavily infected chicks which showed abundant exflagellation and, in a stained smear, usually at least one gametocyte per oil-immersion field of the microscope.

The stock colony of *A. aegypti* has been bred continuously in this laboratory for 8 years. The adults are kept in a large cage supplied with a cotton pad moistened with water and another pad moistened with sugar solution and bearing a few crushed raisins. They are given a blood meal on a guinea pig twice a week. Eggs are laid on filter paper half immersed in a beaker of water. They are not used for breeding purposes until at least a week old. The larvae are reared in tap water to which suitable small amounts of dry powdered yeast and powdered milk are added daily.

The susceptible strain "D," the establishment of which will be described presently, is now maintained in the same manner.

A stock colony of *Aedes albopictus*, derived from eggs which were very kindly sent me by Dr. G. L. Orth of the Army Medical School and Dr. R. L. Laird at Johns Hopkins, is now kept going at a high level by the same methods used for *A. aegypti*.

For the selection experiments, a group of mosquitoes was allowed to feed on a suitable chick. A number of the engorged females were then isolated in half-pint milk bottles, 1 ♀ per bottle. Each bottle contained water to a depth of 2 cm and a circle of 12.5 cm filter paper inserted vertically against the side of the bottle. This filter paper provided a foothold for the mosquito as well as a place on which it could lay its eggs. The bottle was covered with a piece of gauze having in its center a small cotton-plugged hole, through which the mosquito was introduced from the catching tube. A pledget of cotton soaked in sugar solution was placed on top of the gauze. This was moistened daily. Occasionally, a small amount of water had to be added to the bottle to replace loss by evaporation. Female *A. aegypti* isolated under these conditions laid eggs and usually survived the 10–14 days required for the experiments. Since *A. aegypti* eggs, if kept slightly moist, survive but do not hatch until immersed in water, the eggs were left with the parent female until the

latter had been dissected. On the basis of the dissection results, suitable batches of eggs were selected for further breeding.

The stock and experimental mosquitoes were all kept in a greenhouse at temperatures of 30–32° C (usually 30–31° C).

RESULTS

The selection experiments.—In Table 1 are indicated results with all the selections which led to the establishment of strain D, as well as results with the offspring of other related females. A female was considered infected if it showed either oöcysts or sporozoites. The third

TABLE 1.—*Results of selective breeding for susceptibility, starting with stock Aedes aegypti females*

Generation	Group No.	Derived from infected ♀ no.— of group no.—*	Number dissected	Number infected	Per cent infected	Number with oöcysts only
I	1	...	27	3	11	2
II	2†	1:1	20	18	90	3
	3	2:1	13	7	54	4
	4	3:1	10	3	30	1
III	5†	1:2	10	6	60	0
	6	2:2	7	4	57	1
	7	3:2	11	4	37	1
IV	8	4,5,6:2	17	8	47	3
	9†	1:5	15	12	80	4
	10	2:5	20	15	75	5
V	11	1,2,3:8	12	5	42	0
	12	1-8:9 and 1-6:10	28	4	14	0
	13	9:9	6	2	33	0
	14†	10:9	7	5	71	0
	15	11:9	4	3	75	0
	16†	12:9	9	8	90	0
	17	7:10	11	7	63	0
VI	18†	1:14	7	4	57	..
	19	1:15	8	7	88	..
	20	1:16	3	3	100	..
VII	21†	2:16	12	6	50	..
	22†	1:18 and 1:21	3	1	33	..
	23	1:19	9	6	67	..
VIII	24	1:20	4	3	75	..
	25	1:23	8	5	62	..
	26	2:23	2	1	50	..
IX	27	1:24	4	1	25	..
	28	1,2:25 and 1:26 and 1:27	13	12	92	0
		Unselected—				
VIII	29†	from 22	13	12	92	0
		Unselected—				
		from 28	19	17	90	0
X	30					

* The number or numbers to the left of the colon refer to the female or females of the group number given to the right of the colon.

† Indicates the selections which led eventually to strain D.

column of the table gives the source of the parent female (or females) of each group of mosquitoes tested for susceptibility. It will be noted from Table 1 that with the first selected generation (generation II) there was already a marked increase in the percentage of susceptible individuals and further selection did not produce any appreciable further increase. This result is very similar to those obtained by Huff (1929) with *C. pipiens* and *P. catheherium*; by Storey (1932) with the leafhopper *Cicadulina mbila*

and the virus, streak disease of maize; and by Black (1941, 1943) with the leafhopper *Aceratagallia sanguinolenta* and the virus, potato yellow-dwarf. However, if unselected eggs were reared from the earlier generations in the course of the selections, the resulting adults had about the same susceptibility as the original stock, whereas when unselected eggs were reared after VII or IX generations (groups 29, 30 of Table 1) the resulting adults were highly susceptible. It was found in this study, just as Huff had found with *C. pipiens*, that the fertility of the mosquitoes appeared to diminish with continued inbreeding. By generation VI, the fifth selected generation, the eggs laid by several of the engorged, infected females (in a line not shown in Table 1) failed to hatch; and by generation VII there were engorged females which laid no eggs. Many of the viable batches which were laid were very small. The females of group 28 laid fairly numerous eggs, all of which were combined to give group 30 with a total of only about 60 or 70 adults. The females of group 30 were given blood meals on June 18, 21, and 24, 1940. They did not begin to lay eggs until June 23, although the stock *A. aegypti* regularly begin laying eggs $2\frac{1}{2}$ –3 days after their blood meal. Although a fair number of eggs was finally obtained, rather few of them hatched. Enough were reared, however, to give another generation of adults which again laid some eggs. But from these eggs only 2 females and a few males could be reared. One of the females was too weak to feed. The other one took two meals of human blood and laid a small batch of eggs 7 days after the first meal. None of these eggs hatched. Thus 8 generations of inbreeding reduced the fertility to such a point that further mixed breeding could not restore it.

In group 22 the females were kept as long as possible in order to secure a maximum number of eggs. The 3 which were dissected represent only the 3 last survivors, so that the unselected eggs used to give group 29 came from considerably more individuals than these 3. Since the fertility of this line also was obviously going down, selective breeding was discontinued. Eggs from group 29 gave only 12 females which took blood meals. By nursing these along, enough eggs were collected for the establishment of a small colony which was treated just like a stock colony (with freshly reared offspring added once in 10 days) for 6 weeks. Then a batch of females was fed on an infected chick. Out of 29 dissected, 18 (62%) were infected. Eggs from 14 of these infected females were reared, and the adults so obtained (on October 11, 1940) were used to establish a new colony, now designated as strain D. No further selection has since been exercised.

Tests of strain D.—From time to time small groups of females from this colony have been fed on chicks infected with *P. lophurae*, and usually about 40–60% have become infected. In two tests, with chicks which

showed fairly active exflagellation and about 1 gametocyte per field, a considerably lower infection rate was obtained, but in both cases it was higher than in comparable groups of stock *A. aegypti* fed on the same chick at the same time. In one of these experiments none out of 20 stock *A. aegypti* and 4 out of 22 strain D *A. aegypti* were infected. In the other experiment, 1 out of 24 stock *A. aegypti*, 4 out of 22 strain D *A. aegypti*, and 5 out of 16 *A. albopictus* were infected. That the low infection rate obtained in these experiments may have been the result of gametocytes which were insufficient either in numbers or in infectivity is indicated by the unusually low percentage of infected *A. albopictus* obtained in the latter experiment. These were fed on the same chick used in this experiment for the two strains of *A. aegypti*.

The most recent test, conducted in the early part of December 1941 with comparable groups of the two *A. aegypti* strains and of *A. albopictus*, deserves description in detail. Since the larval stage of *A. albopictus* takes a few days longer than that of *A. aegypti*, the eggs of this species intended for the experiment were hatched several days sooner. In this way, the adults of all 3 groups emerged within 3-4 days of each other. They were about 4-7 days old when they were allowed to feed on a duckling which was 4 weeks old and had been infected 5 days previously. From each group, 200 engorged females were isolated. Fifty from each group were dissected 10 days later, and both the stomachs and salivary glands examined. The results are summarized in Table 2. It is obvious

TABLE 2

Mosquitoes used	Number out of 50 which showed						Per cent infected with sporozoites
	Sporozoites	1-5 oöcysts*	5-20 oöcysts	More than 20 oöcysts	Oöcysts but no sporozoites	Oöcysts with black spores	
<i>A. aegypti</i> —Stock ..	15	12	7	3	7	11	30
<i>A. aegypti</i> —Strain D	31	17	15	2	3	28	62
<i>A. albopictus</i>	43	10	12	21	0	0	86

* In all cases, the oöcyst figures include all oöcysts found regardless of size and regardless of whether they contained black spores.

that strain D, after more than a year of nonselective breeding, has retained its character of being considerably more susceptible to infection with *P. lophurae* than the stock from which it was derived. It is less susceptible than *A. albopictus*. Half of the infected *A. albopictus* had more than 20 oöcysts on the stomach wall, while only 2 strain D and 3 stock *A. aegypti* had more than 20. Of the total numbers which showed oöcysts, more of strain D had 5-20 per stomach than of the stock. While the stock

showed 7 abortive infections (oöcysts but no sporozoites), strain D showed only 3 and *A. albopictus* none. It is also of interest that no oöcysts containing black spores were noted in the *A. albopictus* group, whereas the majority of the infected individuals in both other groups had such oöcysts. These results suggest that the mechanism of resistance may be effective not only against the zygotes, as shown by Huff (1934), but also against oöcysts in later stages of development. Very heavy salivary gland infections were shown by 10 stock *A. aegypti*, 20 strain D *A. aegypti* and 36 *A. albopictus*. The salivary glands of these heavily infected individuals had a characteristic fragility and stickiness lacking in uninfected or lightly infected glands, and they could be readily recognized in the course of dissection under a low power dissecting microscope. This has been previously noted by Giovannola (1933) in heavily infected salivary glands of *Anopheles*.

The infection of chicks by sporozoites of P. lophurae.—This has been accomplished repeatedly, using 1- to 2-day-old chicks and sporozoites from either *A. aegypti* or *A. albopictus*. In one experiment a chick fed on by 4 infected mosquitoes showed parasites 15, but not 11, days later. Another chick inoculated intracerebrally with the teased up glands of the same 4 mosquitoes first showed infection at the same time. A chick inoculated intracerebrally with material from one infected *albopictus* showed parasites after 23 but not after 19 days.

Laird (1941) had difficulty in infecting ducklings with sporozoites of *P. lophurae* either by bite or by inoculation of infected *A. albopictus*. He did obtain transmission by both methods, but the infections which resulted were much lighter than those ordinarily obtained by blood inoculation. This fact is considered by Laird and by Wolfson (1941) to indicate that *A. albopictus* may not be a suitable vector for *P. lophurae*. In the present experiments, the infections obtained by sporozoite inoculation were also very light. But before drawing any conclusions it is important to consider the following facts. In routine blood inoculation of ducklings, millions or billions of parasites, suspended in their natural medium (blood) are introduced intravenously. In Laird's experiments with sporozoites, unknown and probably very much smaller numbers of parasites were introduced either into capillaries (by mosquito bite), or intramuscularly (by injection). It has been shown that at least 200 asexual parasites must be inoculated intracerebrally into a 1- to 2-day-old chick to produce an infection apparent 11 days after (Trager, 1941). When the glands of one or a few mosquitoes are enormously diluted with saline, it is altogether likely that rather small numbers of viable sporozoites are actually injected. Hence it is not surprising that chicks inoculated intracerebrally with such material take about 10–15 days to show blood infection. The light infections thus far obtained with sporozoite inoculation

may well be due to the fact that *P. lophurae* in general produces heavy infections only if very large numbers of parasites are injected. It is also pertinent to note that while one asexual parasite of *P. cathemerium* can infect a canary (Stauber, 1939), at least 200 sporozoites of the same species were required to produce infection (Rozeboom and Shah, 1934).

DISCUSSION

The experiments here reported confirm those of Huff (1929, 1930, 1931, 1934), in showing once again, with a different genus of mosquito and a different species of *Plasmodium*, that the individual susceptibility or resistance of a mosquito to infection by malaria parasites is a heritable characteristic. In addition, it has now been possible to establish a "susceptible" strain and to maintain it in large numbers for comparison with the original more resistant stock from which it was derived. Huff concluded (1934) that his data indicated that susceptibility of *Culex pipiens* to *P. cathemerium* behaved like a simple recessive Mendelian character. The data for *Aedes aegypti* do not support such a conclusion. In both cases, a precise genetic analysis is difficult because there is no direct way of knowing the susceptibility of the males. Such a genetic analysis has however been possible with 2 species of leafhoppers which transmit 2 different plant viruses. Here both males and females could be tested for ability to transmit. Storey (1932) developed by selection two races of *Cicadulina mbila*, one able to transmit streak disease of maize and the other unable. The ability to transmit behaved as a simple sex-linked dominant Mendelian character. Black (1941, 1943) similarly obtained 2 races of *Aceratagallia sanguinolenta*, able and unable respectively to transmit potato yellow-dwarf. However, hoppers able to transmit the virus appeared in colonies of the inactive race, even though all their ancestors for 10 generations had failed to transmit. The reverse situation was also obtained, suggesting that ability to transmit is controlled by multiple characters.

Naturally occurring or artificially produced strains of mosquitoes, differentiated on the basis of characters other than susceptibility, have in several instances been tested for their susceptibility to malaria parasites. Tate and Vincent (1934) found no difference in susceptibility to *P. relictum* of an anautogenous English and autogenous Greek, Hungarian, Maltese and cross-bred strains of *Culex pipiens*. *Anopheles elutus* and *A. maculipennis* were equally susceptible to infection with *P. vivax* and *P. falciparum*, the greater importance of *elutus* in the transmission of malaria being the result of its greater preference for human blood (Barber and Rice, 1935). A strain of *A. quadrimaculatus* from Tennessee had the same degree of susceptibility to both *P. falciparum* and *P. vivax* as another strain from Florida (Boyd, 1941). Two strains of

A. quadrimaculatus produced by selection on the basis of larval markings were almost equally susceptible to infection with the monkey parasite *P. cynomolgi* (Coggeshall, 1941b). Thus, so far strains of the same species of mosquito differing in susceptibility to malaria parasites have been obtained only by selection for this particular character.

SUMMARY

By selective breeding from infected females, a strain of *Aedes aegypti* was produced which, after more than a year of mass, nonselective breeding, has maintained its character of being much more susceptible to infection with *Plasmodium lophurae* than the stock from which it was derived.

BIBLIOGRAPHY

- BARBER, M. A. AND RICE, J. B. 1935 Malaria studies in Greece. The malaria infection rate in nature and in the laboratory of certain species of *Anopheles* of East Macedonia. *Ann. Trop. Med. and Parasitol.* **29**: 329-348.
- BLACK, L. M. 1941 Hereditary variation in the ability of the clover leaf hopper to transmit potato yellow-dwarf virus. (Abstract.) *Phytopath.* **31**: 3.
- 1943 Genetic variation in the clover leafhopper's ability to transmit potato yellow-dwarf virus. (In press.)
- BOYD, M. F. 1941 The comparative susceptibility of two strains of *Anopheles quadrimaculatus* to infection with human malaria parasites. *Am. J. Trop. Med.* **21**: 751-753.
- COGGESHALL, L. T. 1938 *Plasmodium lophurae*, a new species of malaria parasite pathogenic for the domestic fowl. *Am. J. Hyg.* **27**: 615-618.
- 1941a Infection of *Anopheles quadrimaculatus* with *Plasmodium cynomolgi*, a monkey malaria parasite, and with *Plasmodium lophurae*, an avian malaria parasite. *Am. J. Trop. Med.* **21**: 525-530.
- 1941b Strains of *Anopheles quadrimaculatus*. Inheritance of color patterns in the larvae of *Anopheles quadrimaculatus*. *Am. J. Trop. Med.* **21**: 755-765.
- GIOVANNOLA, A. 1933 Caratteristiche alterazioni della glandole salivary dell' *Anopheles maculipennis* infetto da parassiti malarigeni. *Riv. Malariol.* **12**: 1140-1146.
- HUFF, C. G. 1927 Studies on the infectivity of plasmodia of birds for mosquitoes, with special reference to the problem of immunity in the mosquito. *Am. J. Hyg.* **7**: 706-734.
- 1929 The effects of selection upon the susceptibility to bird malaria in *Culex pipiens* Linn. *Ann. Trop. Med. and Parasitol.* **23**: 427-440.
- 1930 Individual immunity and susceptibility of *Culex pipiens* to various species of bird malaria as studied by means of double infectious feedings. *Am. J. Hyg.* **12**: 424-440.
- 1931 The inheritance of natural immunity to *Plasmodium cathemerium* in two species of *Culex*. *J. Prev. Med.* **5**: 249-259.
- 1934 Comparative studies on susceptible and insusceptible *Culex pipiens* in relation to infections with *Plasmodium cathemerium* and *P. relictum*. *Am. J. Hyg.* **19**: 123-147.
- 1940 Quantitative studies on size, variability and growth rates of oöcysts of different strains of avian malaria. *Am. J. Hyg.* **32 C**: 71-80.
- HURLBUT, H. S. AND HEWITT, R. 1941 Sporozoites of *Plasmodium lophurae*, an avian malaria parasite, in *Anopheles quadrimaculatus*. *Pub. Health Rep., U. S. Pub. Health Service* **56**: 1336-1337.

- LAYRD, R. L. 1941 Observations on mosquito transmission of *Plasmodium lophurae*. Am. J. Hyg. **34** C: 163-167.
- ROZEBOOM, L. AND SHAH, K. 1934 Preliminary studies on the production of bird malaria infections by the injection of sporozoites. J. Parasitol. **20**: 198.
- STAUBER, L. A. 1939 Factors influencing the asexual periodicity of avian malaras. J. Parasitol. **25**: 95-116.
- STOREY, H. H. 1932 The inheritance by an insect vector of the ability to transmit a plant virus. Proc. Roy. Soc. **112** B: 46-60.
- TATE, P. AND VINCENT, M. 1934 The susceptibility of autogenous and anautogenous races of *Culex pipiens* to infection with avian malaria (*Plasmodium relictum*). Parasitol. **26**: 512-522.
- TRAGER, W. 1941 Studies on conditions affecting the survival in vitro of a malarial parasite (*Plasmodium lophurae*). J. Exper. Med. **74**: 441-462.
- WOLFSON, F. 1940 Successful cultivation of avian plasmodia in duck embryos. Am. J. Hyg. **32** C: 60-61.
- 1941 Avian hosts for malaria research. Quart. Rev. Biol. **16**: 462-473.

OBSERVATIONS ON THE LIFE HISTORY AND TAXONOMIC
RELATIONSHIPS OF THE TREMATODE
*ASPIDOGASTER CONCHICOLA**

CHARLES O. WILLIAMS

In 1827, von Baer described a trematode which he named *Aspidogaster conchicola*. The original material was from a fresh-water mussel of the genus *Anodonta*, from Prussia. This same species has long been known to occur in the pericardial and renal cavities of North American UNIONIDAE, but most of the studies carried on by American investigators have dealt chiefly with morphology and distribution of the species. Kelly (1899) carried on extensive studies of the host relationships of this species in the Illinois River and Stunkard (1917) made a study of the general morphology which confirms and adds somewhat to the earlier observations by Stafford (1896). There has been but fragmentary information concerning the development of this parasite of the UNIONIDAE. Stafford (1896), Faust and Tang (1936), and others have made the suggestion that the life cycle is fundamentally direct, i.e., that the young worm develops directly into an adult, either in the same host individual which shelters the mature worm, or in another individual of the same or of a different species. The systematic position of *Aspidogaster* has long been in doubt. Whether it belongs in MONOGENEA, DIGENEA, or an intermediate group has been a controversial question. It is the object of this paper to trace the life cycle of this worm and to review critically the evidence as to its systematic position.

In the present study, the process of hatching of the eggs of *Aspidogaster* has been observed and, in natural infections, series of immature stages have been found representing fairly complete developmental stages all within the same host species. *Aspidogaster conchicola* therefore differs from the general pattern of host-parasite relationship among trematodes in that the entire life cycle occurs within an invertebrate host.

UNIONIDAE from the Sangamon River near Mahomet, Illinois; from the Salt Fork, at Homer Park, Illinois; and from the Wabash River at West York, Illinois, are the hosts of the *Aspidogaster* used in this study. A detailed list of the host species will be presented in another paper dealing more directly with relationships to the host.

Received for publication, February 8, 1942.

* A contribution from the Zoological Laboratory of the University of Illinois, Urbana. These results, which were in preliminary draft when Ensign Williams was called into the armed services, have been edited by H. J. Van Cleave, under whose direction the research was conducted.

MORPHOLOGY OF ADULT

The elongated body of *Aspidogaster conchicola* is almost entirely covered on the ventral side by a large, alveolated sucker. The body of the worm, exclusive of the ventral sucker, is typically spindle shaped and during locomotion may be stretched to twice its normal length. The highly distensible mouth, located at the end of the neck, is surrounded by an oral sucker. The pharynx, posterior to the oral sucker, is small, but in the living worm it may be seen opening and closing. The intestine, a blind sac which extends almost the entire length of the body, is unbranched but broadens slightly as it continues posteriorly.

The genital pore, located in a slight depression at the union of the ventral sucker and the neck, is on the ventral surface of the neck and at the anterior end of the ventral sucker. A common genital atrium receives both the cirrus, within the cirrus sac, and the uterus. The small ovary is located in the ventral portion of the body. It lies slightly dorsal to the ventral sucker, about midway the length of that organ. The oviduct, leaving the ovary, continues toward the posterior end of the body. It is joined by the Laurer's canal, the paired vitelline canals, and the Mehlis' gland. When filled with eggs, the uterus is tightly coiled back and forth. Upon reaching the posterior end of the body it curves forward toward the anterior end to reach the genital atrium. The unpaired testis, somewhat larger than the ovary, lies along the ventral surface of the body, dorsal to the ventral sucker and posterior to the ovary. The vas deferens leaves the testis posteriorly, then turns anteriorly and continues until it reaches the cirrus sac. Here it empties into the widened seminal vesicle, which ends in the cirrus. The very numerous vitelline follicles are placed laterally in the body. They extend from the posterior end of the cirrus sac to a point just about even with the posterior end of the testis.

The excretory system is one of the most highly developed systems present in the adult *Aspidogaster*. A common excretory pore is present at the posterior end of the worm. This pore receives waste products from two individual excretory bladders whose ducts unite only a short distance before the excretory pore is reached. A single excretory canal empties into each bladder. These canals continue anteriorly and branch out to receive waste products from all parts of the body. Faust (1922: 114) states that there is a total of 243 capillaries and flame-cells for each side of the body of *Aspidogaster conchicola*.

LARVAL DEVELOPMENT

By gradual transformations the larva of *Aspidogaster* acquires the adult stage without sharp metamorphosis to mark off critical, fixed forms. A series of arbitrarily selected steps in the development are described below. The necessity for following this procedure instead of the practice

of describing distinct larval stages is imposed by the fact that larval development is direct.

In observing living adults of *Aspidogaster conchicola*, some of the uterine eggs may be observed to contain active embryos. Faust (1922, Plate XIV) has figured the hatching from the egg membranes of one of these, but the full development has not been traced previously.

First Larval Stage.—Upon the emergence from the egg membranes, the larva (Fig. 1) is not a ciliated miracidium but is a young worm with rudiments of most of the adult structures already discernible. The operculate, embryonated egg is 128 to 134 μ long and 48 to 50 μ wide. Upon hatching, the larva is 130 to 150 μ long and 50 to 55 μ in diameter. The body of the newly-hatched larva as viewed laterally, is generally seen to be divided into three main divisions: anterior, middle, and posterior, each of which contains a primordium of some later structure. These divisions are not separated by actual walls or membranes, but are set off by external grooves.

At the anterior end of the newly hatched larva (first stage) a relatively large oral sucker is recognizable. The oral sucker and the pharynx, extending posteriorly from it, are lined with contractile fibers, for the opening and closing of the sucker and pharynx are common occurrences in newly hatched individuals.

The larval intestine is a cylindrical cavity located in the central division of the body. As a general rule, it is smaller in diameter than the oral sucker. The intestine, together with the excretory bladders which lie on each side of it, at times cause the middle portion of the body to become distended.

In the posterior division of the larva (Fig. 1 VS), the ventral sucker appears as an internal pocket or sac-like organ. In living larvae it contracts and expands. This pocket opens to the ventral surface of the body by a deep cleft which lies just anterior to the small tail-like projection. Surrounding the external opening are muscles which enable the larva to open or close the suckers. At this early stage of development the sub-terminal ventral sucker is capable of becoming attached to objects. Two newly hatched larvae under observation clung together by means of their ventral suckers for about 48 hours. The depth and width of the ventral sucker could not be obtained accurately in living specimens. The larvae were too active for camera lucida drawings, so it became necessary to fix the worms. Alcohol-formol-acetic (85 parts of 85% alcohol, 10 parts formalin, 5 parts glacial acetic) proved a desirable killing agent. However, it was found that with all killing agents the pocket of the ventral sucker was somewhat distorted.

The locomotor ability of newly hatched larvae is negligible. No swimming movements have been noticed. In fact, the suckers of the

newly hatched worm are immediately functional as organs of fixation. After several minutes of activity, larvae under observation have shifted their positions only a few microns. The conclusion may then be reached that either the larvae remain within the viscera of the same host individual throughout their entire life cycle, from egg stage to the adult, or they are transferred passively by water current from host to host.

Second Larval Stage.—Of the numerous immature individuals that have been observed, a second stage larva (Fig. 2) has been recognized, characterized by (1) an increase in size from $150\ \mu$ in length to $275\ \mu$; (2) loss of body constrictions; and (3) attainment of greater powers of locomotion. In this stage there has not yet appeared any conspicuous change in the subterminal ventral sucker.

An adult *Aspidogaster* taken from the pericardial cavity of a mussel, *Anodonta grandis*, had a larva of the second stage, $272\ \mu$ long and $48\ \mu$ wide, clinging to the external lip of the oral sucker. When the larva was detached from the adult and placed on a slide, it moved like a leech, shifting the oral and ventral suckers. Even though this larva was only about $100\ \mu$ longer than the newly hatched larva, its power of locomotion was pronounced, though it lacked ability to swim.

The pharynx and intestine of this larva were more highly developed than those of the newly hatched larvae. The ventral sucker was $64\ \mu$ in diameter, as compared with that of the recently hatched larva which was $40\ \mu$, and the oral sucker was $68\ \mu$ in diameter.

Third Larval Stage.—A third larva taken from the pericardial cavity of a mussel heavily infected with adults, was $880\ \mu$ long and about $180\ \mu$ wide. The oral sucker, when fully extended and attached to the substratum of the container, was approximately $195\ \mu$ in diameter.

The shape of this larva is greatly modified from that of the previously described forms. Instead of the characteristic shape of newly hatched larvae in which the body seems divided into three vague divisions, this later form has lost its constrictions. The oral sucker, more prominent than that of the younger larvae, is located at the end of a long neck-like structure, much as in the adult worm, though the ventral sucker has not yet attained the form and position characteristic of the mature worm.

Posterior to the oral sucker and situated in the neck, is the pharynx, in shape identical with the pharynx of the adult. Just posterior to the pharynx is the sac-like intestine, which extends approximately three-fourths of the body length. In sagittal sections, masses of primordial germ cells were observed in this stage (Fig. 9).

Although the general body shape of this third larval stage is different from that of the newly hatched larvae, there are points of recognizable resemblance. In the first place, the positions of the oral sucker, ventral sucker, intestine, and rudimentary tail are much the same. In the very

young larvae, the ventral sucker is located in the posterior end of the worm, with the rudimentary tail just posterior to it (Figs. 6 and 7). This same arrangement is found in the later larval forms. The ventral sucker of the third larval stage (Fig. 10) bears only a slight resemblance to that of the adult worm. It has assumed larger proportions in this later larval form, for almost the entire posterior fourth of the worm is filled by the large sac-like sucker (Fig. 8) which extends ventrally from the dorsal level of the intestine, opening on the ventral surface (Figs. 6 and 7). The lips of this sucker have a muscular rim and do not spread out over any of the surrounding tissue. As the larva develops, this pocket gradually becomes more shallow and the lips of the pocket begin to extend both anteriorly and posteriorly over the ventral surface of the worm. The greater extension of this outgrowth of the lips is anterior in direction. The body wall seems to have grown down over the increased ventral sucker, for in this stage when the worm is attached by the ventral sucker, the body may be lifted free from the object to which it is attached, being entirely supported by the ventral sucker. In recently hatched larvae (Fig. 1), the entire ventral surface of the worm is in contact with the floor of the containing vessel. In the first and second larval stages, only a circular ring of muscles is seen as the external part of the sucker. The third stage represents a transition toward conditions in the adult worm, where a foot-like ventral sucker has no opening but is made up of numerous alveoli spread over the ventral surface of the body.

Fourth Larval Stage.—A fourth and more advanced larval stage (Fig. 3) was found in the pericardial cavity of a heavily infected mussel. Body shape and structure, as well as the use of external parts, were almost identical with those of the adult. The larva was from 1200 to 1400 μ long when extended and about 320 μ wide. The ventral sucker was about 560 μ long and 320 μ wide. The oral sucker was larger and a little more prominent than in the preceding larval stage just described. It was no longer of major importance in locomotion.

The ventral sucker was in the last stages of metamorphosing from an invaginated pocket-like sucker, of which the greater part was contained inside the body, to the ventral sucker external on the body of the adult (Fig. 4). The position and general outline of the ventral sucker of this larva were identical with those of the adult but the alveoli were not yet fully formed. As the evagination of the ventral sucker continued, the portion spread out over the ventral body surface began to take on the general characteristics of the adult structure. In some individuals of the fourth stage larva, the two median rows of alveoli were not yet formed, but the boundaries of the lateral rows were distinct. Near the posterior end of the ventral sucker, a slit-like opening of a shallow pit was recognized (Fig. 5) as a vestige of the orifice of the deep internal sucking cup

of the earlier larval stages. The opening of the pit did not extend completely across the sucker laterally, but stopped short of the margin of the sucker on both sides. The slit-like opening of a living worm was in the shape of a quarter-moon. The boundaries of the lateral rows of alveoli could be seen extending up to the rim of the pit, then extending very faintly across the bottom of the depression, and finally reappearing on the other side. The posterior rim of the pit did not extend to the posterior end of the sucker, but stopped just short of it. At times the worm would curl the posterior tip of the sucker forward, in this manner deepening and enlarging the pit (Fig. 9). This was noticed especially whenever the worm had lost its anchorage and was securing a new attachment. Crawling in this larva was at a relatively much slower rate than in the previously described stage. The oral sucker no longer assisted in locomotion, except whenever the ventral sucker became detached. Then the oral sucker would fasten itself until the ventral sucker could regain its attachment.

The excretory system of this larva was well developed. Numerous flame cells were observed and the main branches as seen in the adult were present. The large loops of the excretory tubules in the region of the pharynx were particularly evident. These extended posteriorly, one on each side of the body in the region of the attachment of the ventral sucker to the body proper, where each ended in an excretory bladder. These bladders were separated by a much longer partition than are the bladders of the adult. The bladders were connected to the exterior by a very short common canal. At times, muscles in this region contracted

TABLE 1.—*Comparison of various stages in the development of Aspidogaster conchicola. All measurements of living, active worms are expressed in microns*

Stage	Dimensions		Oral sucker diameter	Ventral sucker diameter	Description of ventral sucker
	Length	Diameter			
Adult	2500 to 2750	1150 to 1225	290	554	Composed of four rows of rectangular alveoli. Covers ventral surface.
Embryonated egg	128 to 134	48 to 50			
Stage I	130 to 150	50 to 55	44	40	Deep sac-like organ near posterior end of larva.
Stage II	275	50	68	64	Deep sac-like organ.
Stage III	880 to 960	175 to 180	195	205	Sac-like organ beginning to evaginate. Not as deep as in Stage II.
Stage IV	1200 to 1440	320	275	320	Lateral alveoli appear, median not yet fully formed; only shallow pit remains of sac-like ventral sucker.

and caused a portion of the external body wall to be slightly invaginated. During these periods of contraction the external pore was greatly enlarged and the connections of the bladders with it were visible. After discharge, the bladders were smaller but as excretory products poured in, the bladders again became distended. This distention continued for a time, and then the waste products were forced out through the external pore. The smaller excretory tubules were ciliated and structurally seemed to be identical with those of the adult. Inside the bladders were crystal-like particles, slightly brownish in color, which shifted positions every time the bladders contracted. These particles were not connected to the walls of the bladders, but they were not forced out through the excretory pore.

SYSTEMATIC POSITION OF *Aspidogaster*

The systematic position of the group to which *Aspidogaster conchicola* belongs has been in doubt for many years. The genus has been considered by some workers as belonging to the MONOGENEA; by others, to the DIGENEA (e.g., Ward, 1918); and yet by other investigators (Faust and Tang, 1936: 498–499), as representing a group intermediate in position between the MONOGENEA and the DIGENEA. These worms are in all probability not MONOGENEA, for they lack posterior sucking discs and chitinous hooks or anchors; the excretory pore is single and posterior in position rather than paired anteriorly; and their digestive tract is always rhabdocoele in type. At the same time, there is no evidence that they have an alternation of generations, a prerequisite for typical DIGENEA. Inside the uterine egg is a fully developed embryo, which breaks through the opercular opening to proceed with its development. This larva is not a miracidium, as characteristic for the DIGENEA, for it possesses the fundamental organization of the adult. It develops directly into an adult, either in the same host or in another individual of the same or different species. Parasitized UNIONIDAE when ingested by cold-blooded vertebrates such as fishes, frogs, or turtles, may liberate their worms in the stomach or intestine of the vertebrate. In the worms withstanding digestion, a potential second host becomes annexed to a primitively simple life cycle.

My findings concerning the life cycle of *Aspidogaster conchicola* give added support to the contention of Faust and Tang (1936) that *Aspidogaster* represents a subclass intermediate between the MONOGENEA and the DIGENEA to which the name ASPIDOGASTREA may be applied.

BIBLIOGRAPHY

- VON BAER, K. E. 1827 Beiträge zur Kenntniss der niedern Thiere, in Nova Acta Acad. Nat. Curios 13: 525–557.
FAUST, E. C. 1922 Notes on the excretory system in *Aspidogaster conchicola*. Tr. Am. Mic. Soc. 41: 113–117.

- 1932 The excretory system as a method of classification of digenetic trematodes. *Quart. Rev. Biol.* **7**: 458-468.
- FAUST, E. C. AND CHUNG-CHANG TANG. 1936 Notes on new aspidogastrid species, with a consideration of the phylogeny of the group. *Parasitology* **28**: 487-501.
- KELLY, H. M. 1899 Statistical study of the parasites of the Unionidae. *Bull. Illinois State Lab. Nat. Hist.* **5**: 399-418.
- STAFFORD, J. 1896 Anatomical structure of *Aspidogaster conchicola*. *Zool. Jahrb., Abt. Anat.* **9**: 476-542.
- STUNKARD, H. S. 1917 Studies on North American Polystomidae, Aspidogastridae, and Paramphistomidae. *Illinois Biol. Monogr.* **3**: 1-114.
- WARD, H. B. 1918 Parasitic flatworms, in Ward and Whipple's *Fresh-water Biology*. Wiley, N. Y.

EXPLANATION OF PLATE

All figures are camera lucida drawings representing conditions in *Aspidogaster*. The lines indicating magnification for Figs. 4 to 10 have the value of 132 μ ; for Figs. 1 and 2, 100 μ ; and for Fig. 3, 200 μ .

ABBREVIATIONS

- X—groove in ventral sucker
 I—intestine
 P—pharynx
 Rt—rudimentary tail
 VS—ventral sucker
 OS—oral sucker
 PGS—primordial germ cells

FIG. 1. Newly hatched larva (Stage I); ventral view of stained whole mount in clarite.

FIG. 2. Lateral view of larva (advanced second stage), showing ventral sucker somewhat twisted to left so as to show opening.

FIG. 3. Ventral view of late larva (fourth stage), showing slit-like opening of the shallow pit in the ventral sucker.

FIG. 4. Sagittal section through fourth stage, to one side of median plane, showing location of groove (X) as ventral sucker migrates along the ventral body surface.

FIG. 5. Sagittal section through fourth stage, in median plane, showing location of groove (X) of ventral sucker.

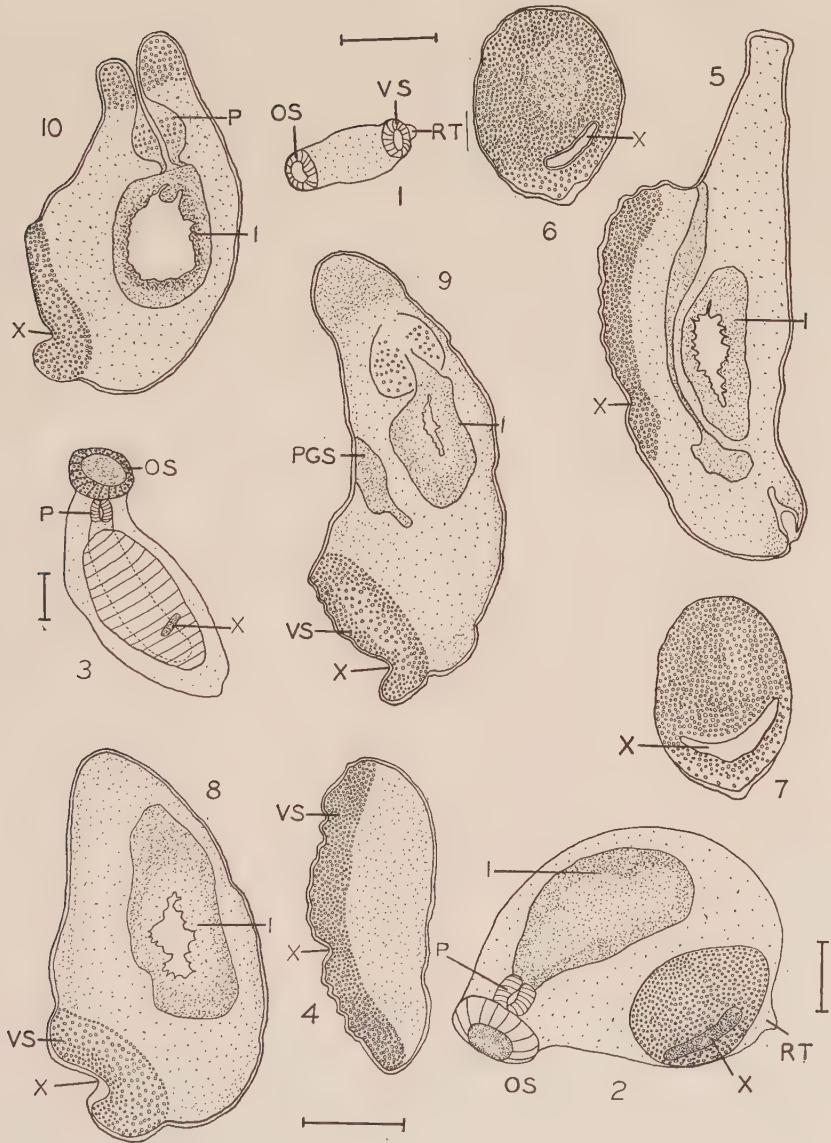
FIG. 6. Frontal section through ventral sucker of advanced second or early third stage, showing shape and position of slit-like opening of ventral sucker pocket.

FIG. 7. Frontal section, same as Figure 6 except that this section is taken ventral to Figure 6 on the same specimen.

FIG. 8. Sagittal section through third stage, showing ventral sucker and slit-like opening; to one side of median plane.

FIG. 9. Sagittal section through third stage, near median plane, showing ventral sucker, slit-like opening of ventral sucker, and posterior tip of ventral sucker which may be curled downward to increase depth of pocket of ventral sucker.

FIG. 10. Sagittal section through third stage, in median plane.



THE USE OF ROENTGEN RADIATION IN LOCATING AN ORIGIN OF HOST RESISTANCE TO *TRICHINELLA SPIRALIS* INFECTIONS*

A. J. LEVIN AND T. C. EVANS

Departments of Zoology and Radiology, State University of Iowa,
Iowa City, Iowa

It has been demonstrated by Ducas (1), McCoy (2, 3), and Fischthal (4), and in experiments included in this investigation, that rats once infected with *Trichinella spiralis* may be highly resistant to subsequent infections. *Trichinella* infections consist of a primary intestinal phase and a secondary muscle invasion. The question has arisen as to whether the host resistance to re-infection is elicited by the penetration of the adult parasites into the intestinal mucosa, the migration of the larvae to the muscles, or both phases. This series of experiments was designed for the purpose of locating the origin of the resistance, and to demonstrate the use of roentgen radiation as a tool in resistance studies.

Tyzzer and Honeij (5), in 1916, Schwartz (6), in 1921, and Semrad (7), in 1937, demonstrated that radium or roentgen irradiation of *Trichinella* larvae, before feeding to a suitable host, would either destroy the larvae before they grew to maturity in the intestine of the host or else prevent the formation of young in the adult female worms that did develop. The extent of injury to the parasites was apparently proportional to the dosage of irradiation. The present authors (8, 9), employing quantitative methods, have determined the range of dosages of radiation which allows the trichina larvae to grow to maturity in the intestine without producing offspring. In this way, an intestinal infection without the consequent muscle invasion could be achieved. The aim of the present experiments is to determine whether an intestinal infection alone will produce resistance to a second infection of trichina.

MATERIALS AND METHOD

Trichinella larvae were removed from infected stock rats by pepsin digestion, and doses of approximately 4,000 larvae for each experimental rat were counted out by the dilution method. These larvae were transferred to small, celluloid dishes and irradiated with a given dose of roentgen radiation, either 3,250 r, 3,500 r, or 3,750 r (H.V.L. ca. 1.5 mm al., 427 r/m), and then fed to rats of the same age, with no previous infections. These were divided into three groups of six rats each, and

Received for publication, February 23, 1942.

* Aided by grants from the Committee on Radiations of the National Research Council and from the Rockefeller Fund for Research on the Physiology of the Cell.

one group of four rats. The group of four rats (Group I) was set up as a re-infection control. These rats received one infection of unirradiated larvae from the same source as the larvae used to re-infect the rats in Groups II, III, and IV.

Two weeks after the initial infection, all of the rats in Group II received re-infections of *Trichinella* which had not been irradiated, to determine whether a resistance to a second infection had developed. Half of the rats received a dose of approximately 2,500 larvae; the other half a dose of about 10,000. One week later (three weeks after the initial infection), the same procedure was carried out with the rats in Group III. On the fourth week after the initial infection, this procedure was again repeated with the rats in Group IV. Two controls (initially infected with unirradiated larvae from the same source as the larvae irradiated for experimental infections) were included in each group.

Two months after the last re-infection, the rats were killed, and dilution counts were made of the larvae removed from the rats' muscles by pepsin digestion.

RESULTS

The first problem to be met in designing these experiments was that of selecting the proper dosage of roentgen radiation. The desired dosage is that which will least affect the development of the *Trichinella* adults in the host intestine, while at the same time inhibit the formation of larvae destined to invade the muscle. Thus, an intestinal infection without the consequent muscle invasion can be accomplished.

After a few preliminary experiments had been performed, it was evident that the proper dosage would be somewhere in the neighborhood of 3,500 roentgens. Several experiments were then carried out using dosages of from 2,000 to 4,000 r, and the results are shown in Table 1. No second infections were given in these experiments. Based upon the evidence in Table 1, it was decided to use three dosages of radiation (3,250 r, 3,500 r, 3,750 r) for re-infection experiments in order to cover possible variations in the *Trichinella* larvae, in the host rats, and in the irradiation. However, it seems that all three dosages were equally effective in creating only intestinal infections.

The data in Table 2 are a summary of the results obtained by re-infecting rats with untreated larvae after they had first been given irradiated ones. Group I includes the re-infection controls, set up to test the viability of the larvae used for re-infection. The ratios given are an expression of the number of larvae recovered from the hosts' muscles divided by the number of larvae that caused the infection.

Group II includes the animals that were re-infected two weeks after the initial infection with irradiated larvae, the dose of radiation being indicated in the next column. The first two cases in this group, where

the initial infection larvae were not irradiated, serve as controls to confirm the findings of Ducas (1), McCoy (2, 3), and Fischthal (4); namely, that one infection causes a resistance to subsequent parasitic invasions. Our results are in accord with those of the above investigators.

In the two control cases marked with double asterisks (**), the low number of larvae recovered from the host muscle may be explained by the fact that the stock rat which served as the source of infective larvae was in the refrigerator for several days before the larvae were digested

TABLE 1.—Data from single infections each with approximately 4,000 irradiated *Trichinella* larvae. Rats of Group I (intestine) were sacrificed 5 days after infection. Rats of Group II (muscle) sacrificed after 2 months

Intestine (Group I)		Radiation dose (roentgens)	Muscle (Group II)	
No. adults recovered	Ratio*		No. larvae recovered	Ratio*
2,970	0.7	0	610,000	152.5
1,970	0.5	0	495,000	123.7
2,200	0.5	0	750,000	163.0
2,110	0.5	0	675,000	162.2
1,629	0.4	0	†	†
1,801	0.4	0	†	†
1,625	0.4	0	†	†
1,900	0.5	0	†	†
2,200	0.5	0	179,000	43.4‡
1,550	0.4	2,000	50,500	12.6
1,475	0.3	2,000	3,100	0.7
2,010	0.5	2,000	2,250	0.6
2,280	0.6	2,000	†	†
2,475	0.6	3,000	564	0.1
700	0.1	3,000	4	< 0.1
2,200	0.5	3,000	23	< 0.1
2,085	0.5	3,000	†	†
1,300	0.3	3,250	1	< 0.1‡
1,900	0.4	3,500	2	< 0.1
196	< 0.1	3,500	3	< 0.1
940	0.2	3,500	†	†
2,700	0.6	3,500	18	< 0.1‡
0	0.0	3,750	8	< 0.1
340	< 0.1	3,750	†	†
1,500	0.4	3,750	9	< 0.1‡
0	0.0	4,000	5	< 0.1
135	< 0.1	4,000	†	†
10	< 0.1	4,000	7	< 0.1

* The ratio expresses the number of worms recovered divided by the number of larvae fed to the rats.

† These rats were destroyed through accident.

‡ The larvae used for infection in these cases were from the same source as those used for the re-infection experiments similarly marked (†) in Table 2.

< 0.1 = less than 0.1.

out. Since these larvae were the only ones available at the time, they had to be used. It seems to have made no difference as far as setting up resistance in the experimental animals. Some of the larvae may have been devitalized, as the number of worms recovered in each of the two cases is small. However, the larvae used for the second infection were not refrigerated and produced a heavy infection in a rat not previously treated (Table 2, Group I, initial infection 9,600). Thus, the small number of worms recovered in these two control cases indicates that the

initial, and not the second infection is reflected in the number of larvae in the muscles. This is in accord with the data compiled by Ducas (1), McCoy (2, 3), and Fischthal (4). In computing ratios of these and other controls in Groups III and IV, Table 2 (0 irradiation), the number of larvae recovered is divided by the number of larvae used for the initial infection (or the larvae causing the infection).

However, in computing the ratios for the experimentals, the number of larvae recovered from the muscles was divided by the number of larvae re-infected because the data from Table 1 indicate that the initial dose

TABLE 2.—Data from re-infection experiments

Group	Initial infection (larvae)	Radiation dose (roentgens)	Re-infection dose (larvae)	No. muscle larvae recovered	Ratio*
I (re-infection control)	9,600			411,000	42.8
	2,500			165,000	66.0
	2,920			(Note a)	...
	4,120‡			179,000	43.4
II (re-infected 2 weeks after initial infection)	3,900	0	9,920	16,000	**
	3,900	0	2,480	44,000	**
	3,900	3,250	9,920	30	< 0.1
	3,900	3,250	2,480	1,017	0.4
	3,900	3,500	9,920	3,682	0.4
	3,900	3,500	2,480	846	0.3
	3,900	3,750	9,920	75	< 0.1
	3,900	3,750	2,480	103	< 0.1
III (re-infected after 3 weeks)	3,900	0	10,000	147,000	37.7
	3,900	0	2,500	149,000	38.1
	3,900	3,250	10,000	115	< 0.1
	3,900	3,250	2,500	27	< 0.1
	3,900	3,500	10,000	23	< 0.1
	3,900	3,500	2,500	230	< 0.1
	3,900	3,750	10,000	2,800	0.3
IV (re-infected after 4 weeks)	3,900	3,750	2,500	58	< 0.1
	4,120‡	0	10,220	157,000	38.1
	4,120‡	0	2,920	276,000	67.0
	4,120‡	3,250	10,220	45,000	4.4
	4,120‡	3,250	2,920	5,200	1.8
	4,120‡	3,500	10,220	847	< 0.1
	4,120‡	3,500	2,920	36,500	12.5
	4,120‡	3,750	10,220	4,000	0.4
	4,120‡	3,750	2,920	30	< 0.1

Note a: This rat died of trichinosis three weeks after infection.

* The ratio expresses the number of larvae recovered from the muscle divided by the number of larvae causing the infection. Refer to Results.

** Explanation of these two cases will be found in Results.

‡ The larvae used for the initial infection in these cases are from the same source as the larvae similarly marked (§) in Table 1.

< 0.1 = less than 0.1.

of irradiated larvae are not responsible for the trichinae recovered from the muscles, provided the irradiation dose is large enough to destroy all the germ cells in each female. This apparently happens with irradiation doses above 3,250 r (Table 1).

The re-infection doses (Table 2) were divided into light (2,500 larvae) and heavy doses (10,000 larvae), to determine if a heavy infection would break down this intestinal resistance. No appreciable difference in resistance against light or heavy doses was noted.

DISCUSSION

In 1916, Tyzzer and Honeij (5) treated *Trichinella* larvae encysted

in muscle with radium and found that these irradiated larvae produced no infection in mice. Schwartz (6), in 1921, irradiated trichina larvae encysted in pork, and then fed the pork to rats. He observed that the irradiation either destroyed the larvae before they grew to maturity in the intestine of the host, or prevented the formation of young in the adult female worms. This was due, he stated, to the fact that the X-rays exerted a selective action on the sex cells of trichina without necessarily affecting the other vital organs. The sex cells of the adult appeared to be atrophied, no spermatozoa were found in the receptaculum seminis of the females, and no evidence of successful copulation could be found.

Semrad (7), in 1937, repeated these experiments, using more exact doses of roentgen radiation. He found that as the dose of radiation was increased, from 400 r to 2,000 r, the average number of larvae in 12 square millimeters of diaphragm decreased markedly. The present authors (8, 9) repeated Semrad's experiments, using a greater range of radiation, and recovery of larvae in all of the muscle tissue. The same trend was found to be true. In addition, it was found that by selecting a certain range of radiation (3,250 r to 3,750 r) it was possible to obtain survival of the larvae in the intestine, and at the same time prevent any material number of offspring from being produced. To determine if an intestinal infection alone could grant host resistance to a second infection, rats (infected with an initial dose of irradiated larvae) were re-infected with untreated larvae. From the data summarized in Table 2, it is evident that a definite resistance to a second infection of *Trichinella* is produced in rats by the initial feeding of irradiated larvae.

It appears that the acquired immunity to certain helminths is directed largely against the intestinal phase of the parasite, and the mechanisms involved may be an increased secretion of mucus and increased peristalsis (McCoy, 3; Ackert, 12). Several workers have failed to demonstrate a passive immunizing action of infected host serum and most of these, McCoy (3), Chandler (10, 14), and Spindler (11), consider the immunity as being localized in the intestine. Other investigators such as Sheldon (15), Sarles and Taliaferro (16), Culbertson and Kaplan (17), Culbertson (18), and Bachman (19) believe that the initial infection produces a generalized immune response characterized by the production of antibodies. While the scope of this paper does not permit a discussion of the different aspects of the subject of immunity, the data presented herein indicate that the passage of larvae through the vascular system and the invasion of the muscles are not necessary for the development of a resistance to a second infection with *Trichinella spiralis*. Further information on the possibility of the production of a generalized immune response might be gained by resistance studies on parabiotic rats in which one of each pair was first infected with properly irradiated larvae.

As a practical application of the procedure employed herein, the authors wish to suggest the possibility of making young pigs resistant to trichinosis (by feeding them with properly irradiated *Trichinella* larvae) as a means of eradicating human infection. Account should be taken of the differences in reaction to the parasite on the part of the pig and rat hosts, so that further careful investigation should be carried on before applying any of the irradiation and larvae doses (as given here) on a large scale. Although the technical difficulties involved present obstacles, the authors do not feel that they are unsurmountable.

The inhibition of the reproductive capacity of parasites by roentgen radiation seems to be a very effective method of studying host resistance mechanisms, and its application to other parasitic forms is worthy of further investigation. It may be noted that Waxler and Herrick (20) have achieved success in immunizing chickens against coccidiosis with this method. Waxler and Herrick speak of this irradiation effect as attenuation, but further work is undoubtedly needed to determine the exact mechanisms involved. Their procedure has certain similarities to the experiments herein reported, and the results might possibly be due to the inhibition of reproductive stages of the parasite following the irradiation.

The suggestions given above are made in the hope that others will take up this work, utilizing roentgen radiation as a tool in the study of host resistance to parasitic infections.

SUMMARY

1. It is possible to treat *Trichinella spiralis* larvae with roentgen radiation before infecting rats, so that the larvae grow to maturity in the intestine but produce no offspring. In this way, an intestinal infection without the consequent muscle invasion can be achieved.
2. This intestinal infection alone will produce strong host resistance to a second infection of *Trichinella*.
3. Since an intestinal infection alone will produce resistance to a second infection, it is concluded that the origin of a mechanism of host resistance to *Trichinella* is located in the intestine.
4. Theoretical and practical considerations of intestinal resistance are discussed, and suggestions are made for further investigations.
5. The use of roentgen radiation as a tool for the study of host resistance to parasitic infections is urged.

BIBLIOGRAPHY

1. DUCAS, R. 1921 L'immunité dans la trichinose. These, Paris, Jouve et Cie. P. 47.
2. MCCOY, O. R. 1931 Immunity of rats to reinfection with *Trichinella spiralis*. Am. J. Hyg. 14: 484-494.

3. ————1940 Rapid loss of *Trichinella* larvae fed to immune rats and its bearing on the mechanism of immunity. *Am. J. Hyg.* **32** D: 105-116.
4. FISCHTHAL, J. H. 1938 Intestinal infestation with *Trichinella spiralis*. Thesis, State University of Iowa. (Unpublished.)
5. TYZZER, E. E. AND HONEIJ, J. A. 1916 The effects of radiation on the development of *Trichinella spiralis*. *J. Parasitol.* **3**: 43-56.
6. SCHWARTZ, B. 1921 Effects of x-rays on trichinae. *J. Agr. Res.* **20**: 845-854.
7. SEMRAD, J. E. 1937 Effects of roentgen radiation on trichinosis in the albino rat. *Am. J. Roentgenol. and Rad. Ther.* **38**: 470-477.
8. LEVIN, A. J. AND EVANS, T. C. 1940 Effect of roentgen radiation on embryonic development of *Trichinella spiralis*. *J. Parasitol.* **26** Suppl: 31.
9. EVANS, T. C., LEVIN, A. J. AND SULKIN, N. M. 1941 Inhibition of embryo formation in certain nematodes by roentgen radiation. *Proc. Soc. Exper. Biol. and Med.* **48**: 624-628.
10. CHANDLER, A. C. 1932 Susceptibility and resistance to helminthic infections. *J. Parasitol.* **18**: 135-152.
11. SPINDLER, L. A. 1934 Resistance of rats to superinfection with *Nippostrongylus muris*, following administration of adult worms by duodenal tube. *J. Parasitol.* **20**: 326.
12. ACKERT, J. E. 1942 Natural resistance to helminthic infections. *J. Parasitol.* **28**: 1-24.
13. ANDERSON, C. V. AND LEONARD, A. B. 1940 Immunity produced in rats by the intestinal phase of *Trichinella* infection. *J. Parasitol.* **26** Suppl: 42.
14. CHANDLER, ASA C. 1935 Studies on the nature of immunity to intestinal helminths. I. The local nature of the immunity of white rats to *Nippostrongylus* infection. *Am. J. Hyg.* **22**: 157-168.
15. SHELDON, A. J. 1936 Studies on active acquired resistance, natural and artificial, in the rat to infections with *Strongyloides ratti*. *J. Parasitol.* **22**: 533.
16. SARLES, M. P. AND TALIAFERRO, W. H. 1936 The local points of defense and the passive transfer of acquired immunity in experimental trichiniasis. *J. Infect. Dis.* **59**: 207-220.
17. CULBERTSON, J. T. AND KAPLAN, S. S. 1937 Passive immunity in experimental trichiniasis. *J. Parasitol.* **23**: 560.
18. CULBERTSON, J. T. 1942 Passive transfer of immunity to *Trichinella spiralis* in the rat. *J. Parasitol.* **28**: 203-206.
19. BACHMAN, G. W. 1929 A precipitin test in experimental trichiniasis. *J. Prev. Med.* **3**: 465-469.
20. WAXLER, S. H. AND HERRICK, C. W. 1941 Immunization against coccidiosis by the use of x-ray attenuated oöcysts. *J. Parasitol* **27** Suppl: 17.

REMARKS ON THE TAXONOMY OF SOME AMERICAN
CHIGGERS (TROMBICULINAE), INCLUDING
THE DESCRIPTIONS OF NEW
GENERA AND SPECIES

H. E. EWING

Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture

Chiggers are parasitic larvae of mites of the subfamily TROMBICULINAE and infest vertebrates exclusively. They are distributed throughout most of the temperate and tropical regions of the world. Up to the present there have been reported from the Western Hemisphere 10 valid genera and 76 valid species. To this number there are here added four genera, two of which are new, and seven species, all being new. There probably remain to be described from the New World at least twice as many genera and species as have been already reported.

THE PROPER SCIENTIFIC NAME FOR THE COMMON NORTH
AMERICAN CHIGGER

For many years the common North American chigger was known by the scientific name *Leptus irritans*, given to it by C. V. Riley (1873). It was pointed out by Oudemans (1937), however, that the name *Leptus irritans* had been used previously by Lucas (1847) for a "patatta" mite, or chigger, occurring in Brazil. Following this disclosure the writer (Ewing, 1938a, p. 26) reviewed the synonymy of the species and came to the conclusion that *alfreddugèsi* (Oudemans) was the oldest valid name for *irritans* Riley.

When the writer's key to the genera of chiggers was published (Ewing, 1938b), *alfreddugèsi*, which had been described under the generic name *Microtrombidium* and which for some years had been placed in *Trombicula*, was made the type species of a new genus, *Eutrombicula*. Thus the scientific name of our most common and best known chigger became *Eutrombicula alfreddugèsi* (Oudemans). But Oudemans has recently proposed *Leptus rileyi* (Oudemans, 1939) as a new name for *Leptus irritans* Riley. This not only was unnecessary, since there already existed several synonyms of Riley's species, but became confusing since some workers adopted his new name as the proper one to apply to Riley's *irritans* at the present time. If Oudemans were correct in not recognizing his *alfreddugèsi* as being the same as Riley's *irritans*, then the proper name to apply to the latter species would probably be my *cinnabaris* (Ewing, 1921, p. 387) which undoubtedly is the same as Riley's *irritans* but may not have priority over Hirst's *similis* (Hirst, 1921). Although

the printed date for *cinnabaris* is December 1920, the actual date of publication was some time in 1921. The writer sees no good reason for not using the specific name *alfreddugèsi* for Riley's *irritans*.

THE STATUS OF *Trombicula hirsti* SAMBON

Sambon (1927) described under the above name a man-infesting chigger from North Queensland, Australia, known locally as "the scrub itch-mite." According to the description and excellent drawings (made by Terzi) of this species it agrees with our North American *alfreddugèsi* in all respects except one, i.e., the inner prong of the palpal claw in *hirsti* is stouter than the outer, while in *alfreddugèsi* the reverse is true.

Womersley (1939) regards Sambon's species as only a synonym of *Trombicula minor* Berlese, the genotype of *Trombicula*. He states: "I have now received from Dr. W. G. Heaslip an adult female (of *Trombicula hirsti* v. *buloloensis* Gunther) found at Innisfail in Queensland, December, 1939, which also corresponds to Berlese's species (*Trombicula minor*). As the only larval *Trombicula* known from Queensland is *T. hirsti* Sambon, the common itch-mite of that State, the above correction is further confirmed." If this synonymy is correct, *Eutrombicula* Ewing becomes a synonym of *Trombicula* Berlese, and the Japanese chigger, *Trombicula akamushi* (Brumpt), and the autumnal chigger of Europe, *Trombicula autumnalis* (Shaw), belong to *Leptotrombidium* Nagayo and other authors, since both species have a trifurcate palpal claw.

A QUILL-INFESTING CHIGGER

Torres and Braga (1938) have described a Brazilian chigger of unusual habits. This chigger, which has the common name of "micuim", was placed in a new genus, *Apolonia*, and described as new, being given the name *tigipioensis*. According to the authors, the chigger enters the quills of very new feathers of chickens and produces nodules and lesions. They give a photograph of a fowl showing the presence of these nodules on the upper part of each leg.

Torres was in Washington recently and left with this writer a sample of one of these nodules and many infested quills for study. The nodule was about one-half the size of a pea and each infested quill had broken off near its base. In the short, broken-off, basal part of each was found a single chigger. The chigger usually completely filled the lumen of the quill at the latter's base, and always was oriented so as to face the basal end of the quill.

Several of the specimens left by Torres have been cleared and mounted in a modified Berlese mixture. They exhibit the essential taxonomic characters of *Apolonia tigipioensis*, as given by Torres and Braga. If this quill-infesting chigger spreads to other countries, it may become a poultry pest of major importance.

THE NATURAL AFFINITIES OF *Apolonia*

In a second paper, Torres and Braga (1939) have placed *Apolonia* next to *Leeuwenhoekia* Oudemans in a key adapted from Ewing (1929, p. 22). In this they are correct, according to the opinion of the writer. Even in the revised key to genera (Ewing, 1938b), this new genus should be placed next to *Leeuwenhoekia*. It differs from the latter particularly in the loss of the posterolateral setae of the dorsal plate.

Paraschöngastia

Womersley (1939) proposed a new genus, *Paraschöngastia*, for four New Guinea species of *Neoschöngastia*, but failed to name a genotype. According to his diagnosis of *Paraschöngastia*, the contained species differ from the remaining ones of *Neoschöngastia* particularly "in that there is a distinct raised crest in front of the pseudostigmata." Some of our American species of *Neoschöngastia* are very close to those included in *Paraschöngastia*.

NEW GENERA AND SPECIES

There are here described two new genera and seven new species of chiggers. One of the new species, *Schöngastia lynni*, probably will be made the type species of a new genus in the future, when more material is obtained. All the specimens of this species at hand are injured, in that the pseudostigmatic organs have been detached.

Schöngastia lynni n. sp.
(Figs. 1, 2)

Chelicera obliquely flattened at distal end so as to form a posteriorly directed process which is toothed on its margin; ventral tooth large, flattened, directed backward. Palpus not肘ed but rounded laterally; first seta with 2 to 4 short, lateral branches; second seta either simple or with a single, short, lateral branch; palpal claw slightly cleft distally, forming 2 small, sharply pointed, lateral prongs closely appressed to the much larger middle element. Eyes of a side situated on a poorly sclerotized ocular plate; anterior eye slightly smaller than posterior and either granular or pigmented underneath cornea. Dorsal plate about twice as broad as long and shaped as shown in Fig. 2; setae all slightly curved and indistinctly barbed, the median and posterolateral subequal and longer than the anterolateral; pseudostigmatic organs detached from each specimen. Dorsal abdominal setae about 30, slightly curved, indistinctly barbed. The shoulder pair present, followed by a transverse row of 6, then a transverse row of 4, the remainder not arranged in distinct rows. Legs of moderate length, each composed of 7 segments and provided with the usual tarsal armature.

Length of partly engorged larva, 0.63 mm; width, 0.41 mm.

Type host.—"On frog."

Type locality.—Blue Mountains, Jamaica, West Indies.

Type slide (holotype).—U. S. N. M. No. 1412.

Description based on four specimens taken by Dr. Lynn. This species has the chelicerae of *Hannemania* and the dorsal plate of several other genera. It would have been assigned a new genus if the pseudostigmatic

organs had not been detached from every specimen. The writer has been very reluctant regarding the establishment of a new genus upon a species all known representatives of which are without a certain structure of generic value. In the case of *S. lynni*, the loss of the pseudostigmatic organs deprives us of one of the most important generic characters and makes it impossible to place a new genus, established on it, properly in a key.

Trombicula rohweri n. sp.

(Fig. 3)

Chelicera short, stout; dorsal and ventral tooth each very sharp and barblike. Palpus subangulate laterally; first and second palpal setae plumose; inner prong of palpal claw usually more slender, less curved, and more closely appressed to middle one than outer prong; middle prong longer and stouter than the other 2. Eyes 2 on each side, subequal, situated on a thick, strongly sclerotized, ocular plate; anterior eye with granulations and pigment. Dorsal plate (Fig. 3) minutely pitted except along anterior margin and about the base of median seta; behind each pseudostigma is a transverse ridge which may possibly be accompanied by a concealed transverse slit. Dorsal abdominal setae 36 to 40, curved, weakly plumose, arranged in irregular transverse rows. Legs of medium length, seven-segmented; coxae each with enlarged and heavily sclerotized distal margin.

Length of partly engorged larva, 0.47 mm; width, 0.30 mm.

Type host.—Eastern house wren, *Troglodytes aedon aedon*.

Type locality.—Hatsboro, Mississippi.

Type slide (cotypes).—U. S. N. M. No. 1413.

Description based on four cotypes taken February 14, 1940, by G. G. Rohwer.

This species is closely related to *T. oregonensis* Ewing, from which it differs in having a much narrower dorsal plate and a transverse ridge behind each pseudostigma. It differs from *T. californica* n. sp., in having the first palpal seta plumose instead of simple.

Trombicula californica n. sp.

(Figs. 4, 5)

Chelicera short, stout; dorsal and ventral tooth each a minute barb. Palpus not angulate laterally; first and second setae rather short, simple; outer prong of palpal claw usually stouter than inner and not so closely appressed to the middle prong, which is the largest and most strongly curved of the three. Eyes 2 on each side; situated on ocular plate; anterior eye slightly smaller than posterior and somewhat granular under cornea. Dorsal plate slightly broader than long and shaped as shown in Fig. 4; almost the entire surface minutely pitted. Dorsal abdominal setae about 40, slightly curved, weakly plumose; 10 in the first and 10 in the second transverse rows. Legs rather short, each composed of 7 segments and provided with the usual tarsal armature.

Length of partly engorged larva, 0.40 mm; width, 0.28 mm.

Type host.—*Thomomys neglectus*.

Type locality.—Acton, California.

Type slide (cotypes).—U. S. N. M. No. 1414.

This species belongs to the *whartoni* group and is most nearly related to *Trombicula oregonensis* Ewing, from which it differs in having the first

palpal seta simple instead of branched and the dorsal plate slightly broader than long instead of about twice as broad as long. California material at hand as follows: Seven specimens from type host and type locality, April 12, 1933, taken by A. G. Barr; nine specimens on *Thomomys* species, Fresno, July 6, 1932, taken by B. P. Bole, Jr.; four specimens on *Microtus californicus sanctidiegi* and two specimens on "wood rat," Los Angeles County, January 9, 1933, taken by M. F. Canova. British Columbia material as follows: One specimen on *Peromyscus* species, Vancouver, January 14, 1937, from G. J. Spencer.

Comatacarus n. g.

Chelicera with but a single dorsal and a single ventral tooth; chelicera not obliquely flattened distally. Palpus with claw two- or three-pronged, either the first or second seta, or both, plumose or barbed. Eyes present. Dorsal plate entire, moderately sclerotized; crista absent; anterior median process present; pseudostigmatic organs flagelliform, simple; anterolateral setae large, plumose; posterolateral setae not clavate but similar in form to anterolateral; median seta absent; paramedian setae present, similar to anterolateral setae; lateral setae absent. Dorsal abdominal setae similar, sessile, curved, weakly plumose and numerous. Tarsi each armed distally with 3 claws, the outer 2 being equal, strongly curved and stouter than the more slender, longer, and less curved median claw.

Type species.—*Comatacarus americanus* n. sp.

Comatacarus is erected to include a few species which have the characters of *Leeuwenhoekia* Oudemans (1911), it being desired to divide Oudemans' genus into three genera. It differs from *Leeuwenhoekia*, sensu strictu, in having the posterolateral setae of the dorsal plate of the usual form and similar to the anterolateral, instead of being clavate, and in having a large number of sessile setae on the dorsum of the abdomen, instead of a small number situated on the tubercles. Besides the type species, one other from the New World is included, *C. occidentalis* n. sp. Probably one or more of the Old World species will later be referred to this genus.

Comatacarus occidentalis n. sp.

(Fig. 6)

Chelicera narrowed distally and formed into a more or less dartlike head. Palpus not angulate laterally; first seta weakly plumose; second seta simple; palpal claw two-pronged, outer prong much more slender, slightly shorter, and less curved than inner. Eyes equal, almost contiguous, each with a well-formed cornea. Dorsal plate smooth, about twice as broad as long; anterior median process angulate apically; submedian setae weakly plumose, almost straight, and slightly shorter than similar anterolateral setae; posterolateral setae similar to anterolateral but much longer; pseudostigma situated on a short, transverse ridge; pseudostigmatic organ long, simple, flagelliform. Dorsal abdominal setae curved, weakly plumose, and rather closely studding the dorsum, over 80 in number, and arranged in somewhat irregular rows. Legs six-segmented; coxa II more slender than other 2.

Length of unengorged larva, 0.32 mm; width, 0.19 mm.

Type host.—"On common ground squirrel."

Type locality.—Lake Pillsbury, California.

Type slide (holotype).—U. S. N. M. No. 1415.

Description based on holotype, the only specimen at hand, collected September 10, 1939, by Deonier, Bishopp and Lindquist. This species is easily distinguished from its congener, the description of which immediately follows, by having the second palpal seta simple instead of weakly plumose.

Comatacarus americanus n. sp.

(Fig. 7)

Chelicera stout, strongly upcurved at and near apex; dorsal and ventral tooth small, each situated nearer than usual to apex of chelicera. Palpus rather short, rounded laterally; first seta curved, plumose; second seta straight, longer than first, plumose; palpal claw three-pronged, middle prong very much stouter and somewhat longer than the very sharp, almost straight, subequal lateral prongs. Anterior and posterior eyes contiguous, situated on a short, broad, oval ocular plate. Dorsal plate smooth; other characters as shown in Fig. 7. Dorsal abdominal setae more than 80, slightly curved, plumose, 12 setae in first transverse row. Legs six-segmented; coxa II longer but not so broad as coxa III.

Length of partly engorged larva, 0.41 mm; width, 0.27 mm.

Type host.—"Western mole."

Type locality.—Portland, Oregon.

Type slide.—U. S. N. M. No. 1416.

Description based on two lots: Six specimens from type host and type locality, May 20, 1936, taken by C. M. Gjullin and one specimen on "cotton mouse," Dale County, Alabama, April 5, 1937, from R. E. Dyer.

Acomatacarus n. g.

Most nearly related to *Comatacarus* but differing from it as follows: Chelicera with a row of teeth on upper margin instead of a single tooth and both the first and second palpal setae simple instead of one or both being plumose or barbed.

Type species.—*Acomatacarus arizonensis* n. sp.

Of the New World chiggers only the type species is included. Of the Old World species several should be included, three of which are *Leeuwenhoekia polydiscum* Oudemans, *L. jaegerskioeldi* Oudemans, and *L. australiensis* Hirst.

Acomatacarus arizonensis n. sp.

(Figs. 8, 9)

Chelicera rather slender, bearing above from 3 to 5 unequal teeth. Palpus rounded laterally; first and second setae subequal, curved; palpal claw strongly curved and unequally divided, outer prong much smaller and more sharply pointed than inner. Eyes not situated on ocular plate, anterior slightly larger than, and almost contiguous with, the posterior. Dorsal plate (Fig. 9) very broad, smooth. Pseudostigma peculiar in that it is somewhat overlapped in front by a liplike ridge. Dorsal abdominal setae between 40 and 50, rather short, curved, very weakly plumose, arranged in indefinite transverse rows. Legs of medium length, six-segmented, although segments II to V (inclusive) each possesses a pigmented band indicating a false division.

Length of unengorged larva, 0.22 mm; width, 0.12 mm.

Type host.—A lizard, *Callisaurus draconoides ventralis*.

Type locality.—Near Wickenburg, Arizona.

Type slide.—U. S. N. M. No. 1417.

Description based on six specimens taken from type host at type locality, May 11, 1937, by Roy Komarek.

Walchia americana n. sp.
(Figs. 10, 11)

Chelicera stout; upper tooth larger than usual, not recurved or barblike; lower tooth directed backward, acutely angulate at apex. Palpus short, subangulate laterally; first seta short, slightly curved, simple; second seta subequal and similar to first; 3 prongs of palpal claw similar, but middle prong usually larger than others. Eyes absent. Dorsal plate smooth; a short, curved ridge in front of each pseudostigma; pseudostigmatic organs missing from all specimens, probably being detached by the treatment in caustic before mounting; for other characters of dorsal plate see Fig. 11. Dorsal abdominal setae about 26, very slightly curved, each with a few minute lateral branches or barbs. Number of setae in first 4 transverse rows as follows: 6, 6, 4, 4. Legs very short; those of first pair seven-segmented (femur divided), those of other pairs six-segmented; coxa II longer and more slender than other 2.

Length of unengorged larva, 0.24 mm; width, 0.13 mm.

Type host.—"On cotton mouse."

Type locality.—Tallahassee, Florida.

Type slide (cotypes).—U. S. N. M. No. 1418.

Description based on nine specimens taken on type host from type locality, November 8, 1936, by B. V. Travis. This species is placed in *Walchia* with some hesitation. It is closely related to *Gahrlepiea turmalis* Gater of the Federated Malay States, from which, however, it differs in having the dorsal plate of a different shape. Like *G. turmalis*, *W. americana* has the first and second palpal setae short, curved, and simple, and femur I divided, yet unlike *G. turmalis* it has a short, curved ridge near each pseudostigma as is the case in *Walchia lewthwaitei* Gater.

BIBLIOGRAPHY

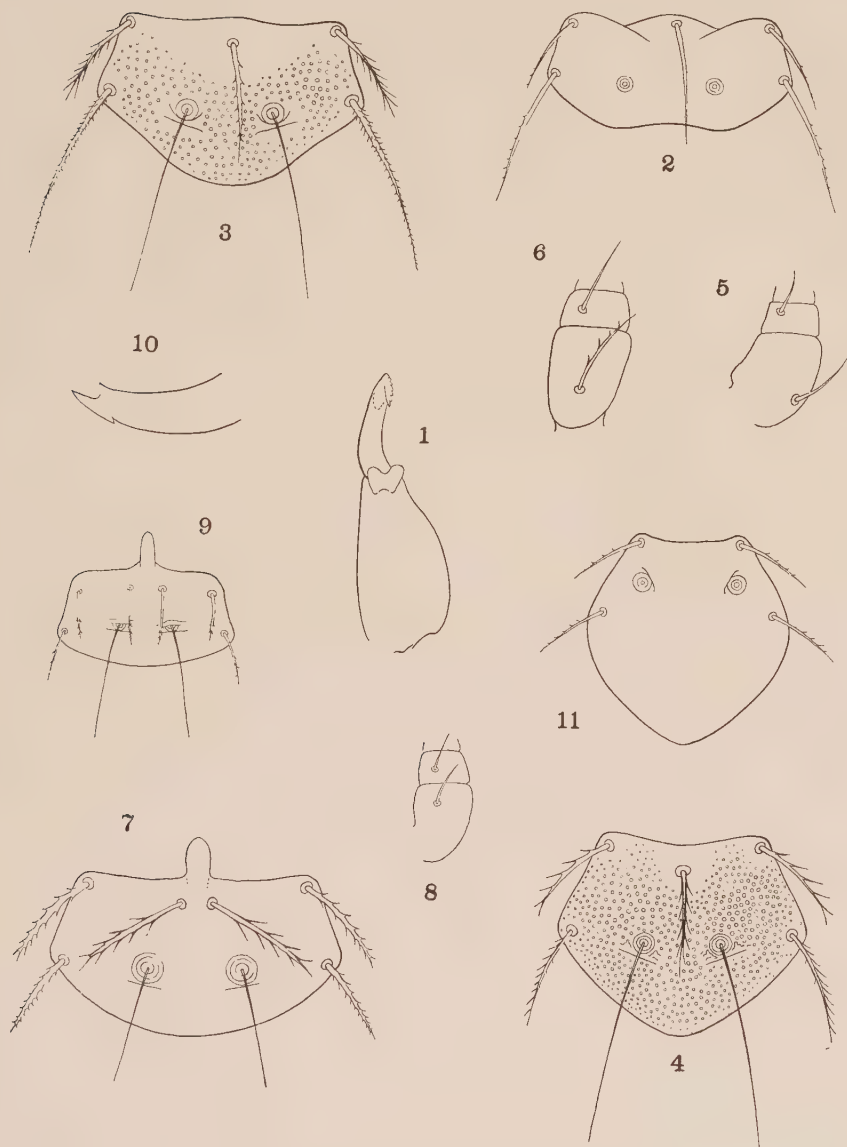
- EWING, H. E. 1921 The genus *Trombicula* Berlese, in America and the Orient. Ann. Entom. Soc. Am. 13: 381-390.
 ——— 1929 A Manual of External Parasites. Charles C. Thomas, Springfield, Ill. 225 pp.
 ——— 1938a The scientific name of the common North American chigger preoccupied. Proc. Helm. Soc. Washington 5: 26-27.
 ——— 1938b A key to the genera of chiggers (mite larvae of the subfamily Trombiculinae) with descriptions of new genera and species. J. Wash. Acad. Sc. 28: 288-295.
 HIRST, S. 1921 On three new parasitic mites (Leptus, Schöngastia, and Demodex). Ann. and Mag. Nat. Hist. (9)7: 37-39.
 LUCAS, H. 1847 Sur des Acariens Trouvés au Para. Bul. Soc. Entom. France (2)5: xxxvi-xxxvii.
 OUDEMANS, A. C. 1937 Kritisch Historisch Overzicht der Acarologie. Pt. 3, Vol. D, p. 1389.
 ——— 1939 Neue Funde auf dem Gebiete der Systematik und der Nomenklatur der Acari. VI. Zool. Anz. 127: 75-80.
 RILEY, C. V. 1873 Harvest mites. Am. Naturalist 7: 16-19.
 SAMBON, L. W. 1927 The "scrub itch-mite" of North Queensland: a new species of *Trombicula*. Ann. and Mag. Nat. Hist. (9)20: 157-161.
 TORRES, S. AND BRAGA, W. 1938 Nova parasitose em pintos. Trab. Sec. Veter. Inst. Pesq. Agron. Pernambuco, pp. 171-172.

- AND ——— 1939 *Apolonia tigipiöensis*, g. et sp. n. (Trombiculinae) parasito de *Gallus gallus* dom. Bol. Soc. Bras. Med. Vet. **9**: 1-7.
- WOMERSLEY, H. 1939 Further notes on the Australian Trombidiidae with descriptions of new species. Tr. and Proc. Roy. Soc. South Australia **63**(2): 149-166.

EXPLANATION OF PLATE

All drawings of the dorsal plate are of the same magnification, the others are differently and more highly magnified.

- FIG. 1. *Schöngastia lynni* n. sp.; dorsal view of chelicera.
- FIG. 2. *Schöngastia lynni* n. sp.; dorsal view of dorsal plate. (Pseudostigmatic organs probably detached by process of clearing specimens.)
- FIG. 3. *Trombicula rohweri* n. sp.; dorsal view of dorsal plate.
- FIG. 4. *Trombicula californica* n. sp.; dorsal view of dorsal plate.
- FIG. 5. *Trombicula californica* n. sp.; dorsal view of basal part of palpus.
- FIG. 6. *Comatacarus occidentalis* n. sp.; dorsal view of basal part of palpus.
- FIG. 7. *Comatacarus americanus* n. sp.; dorsal view of dorsal plate.
- FIG. 8. *Acomatacarus arizonensis* n. sp.; dorsal view of basal part of palpus.
- FIG. 9. *Acomatacarus arizonensis* n. sp.; dorsal view of dorsal plate.
- FIG. 10. *Walchia americana* n. sp.; side view of piercing arm of chelicera.
- FIG. 11. *Walchia americana* n. sp.; dorsal view of dorsal plate.



RESEARCH NOTES

THE MOURNING DOVE, A NEW HOST OF THE ANOPLOCEPHALID TAPEWORM, *APORINA DELAFONDI* (RAILLIET)

Thirty-one mourning doves, *Zenaidura macroura carolinensis* (L.), from Wake County, North Carolina, were examined for parasites during the spring of 1940. Five of them were infected with mature specimens of the tapeworm, *Aporina delafondi* (Railliet). One host harbored three specimens and the other four were parasitized with one each. This report appears to be the first record of this species infecting the mourning dove. It has been recorded by Cram (1924, J. Parasitol. 11: 115) and Hussey (1941, Am. Midland Naturalist 25: 413-417) as infecting the pigeon in the United States.—REINARD HARKEMA, Zoology Department, North Carolina State College, Raleigh, N. C.

AN IMPROVED TECHNIQUE FOR THE STUDY OF THE ACANTHOR STAGE IN CERTAIN ACANTHOCEPHALAN LIFE HISTORIES

Manter (1928, Tr. Am. Micr. Soc. 47: 342-347) described a simple method by which he was able to induce artificial hatching of acanthocephalan eggs. By allowing a culture of eggs, from the body cavity of a mature female worm, to dry completely in a Syracuse watch glass and then re-wetting with water he found that a number of eggs hatched immediately, releasing the first larval stage, the acanthor. Manter used the method successfully with the eggs of *Macracanthorhynchus hirudinaceus* and *Mediorhynchus* sp., but he was not able to induce the artificial hatching of the eggs of *Neoechinorhynchus cylindricus*.

The author has increased the efficiency of Manter's method by several modifications. Instead of placing the eggs in a Syracuse watch glass to dry, a smear of eggs from the body cavity of a female worm, not more than one layer thick, is made on a clean glass slide, and allowed to become completely desiccated, either by drying in an incubator at 37.5° C for 3-4 hours or drying at room temperature overnight. The smears may then be stored in a covered slide-box until examined. In the humid Houston climate artificial hatching has been observed after storage for more than a month. However, the number of eggs that will hatch by this method decreases as the storage time is increased. In place of plain water for re-wetting the desiccated smears the author uses a dilute solution of neutral red (10 drops of a saturated solution in 20 cc of neutral distilled water). This re-wetting with the dilute vital stain induces artificial hatching just as well as plain water, and has the advantage of staining the larvae. A coverglass over the smear permits the use of the high dry and oil immersion objectives for the study of the acanthor stage. The central nuclear mass and the hypodermal nuclei of the acanthor stain a deep red. The presence of the stain in the tissue of the acanthor accentuates the outline of the subcuticular muscles and the retractor muscles of the rostellum. These muscles are invisible in unstained specimens.

The author has confirmed Manter's observation that only eggs from the body cavity of a mature female worm may be induced to hatch in this manner. Eggs from the feces of infected animals fail to hatch.

The author has been successful in hatching the eggs from the body cavities of the following Acanthocephala in this manner: *Moniliformis dubius*, *Macracanthorhynchus hirudinaceus*, *Macracanthorhynchus ingens*, *Hamanniella tortuosa*, and *Mediorhynchus grandis*. Attempts to hatch the eggs of *Neoechinorhynchus emydis* and *Centrorhynchus* sp. by this method have proved unsuccessful.—DONALD V. MOORE, Rice Institute, Houston, Texas.

NOTICE TO MEMBERS, SUBSCRIBERS, AND AUTHORS

The war effort must of necessity have its repercussions in the problem of publishing a scientific journal, especially one so exclusively dependent for its publication funds on income from members and subscribers, as is the case with the *JOURNAL OF PARASITOLOGY*. It was to be expected that foreign subscriptions would be reduced sharply in number, reductions which have been augmented by export prohibition to some countries. Individual energy needs to be used to compensate for these losses, not only by maintenance of membership in the Society, but by personal effort to increase domestic circulation. When inadequate resources are available for publishing, one effect is to postpone, in a disheartening way, the printing of acceptable articles.

During the year the Editorial Committee and Editorial Board have been examining possibilities which would tend to balance a net budget that is experiencing decrease both through lessened regular income and increased costs. They have decided to undertake, in experimental mood, two measures which will immediately strike the eye of the reader of Volume 29, and which will reduce to about 80 pages what requires 100 now. One of these is to use a slightly larger type page, holding the overall size of the *JOURNAL* page as at present. The other is to utilize more of the space, which for reasons of economy of effort in both the editorial office and the press, has hitherto been blank. Also to be mentioned is the omission of a separate index to the Supplement in this December issue. As the Supplement carries its own author index and the program is essentially a table of contents, the details of the compactly printed abstracts are considered readily accessible. These three methods of rationing of space will produce savings. Others may become necessary.

As a further contribution to the general problem, authors are urged to give a new quality of attention to the job of saying more in fewer words: shorter introductions, more compact factual presentations, briefer discussions, decreased overall lineage between the title and last bibliographic item. It would be a contribution to more than the war effort if articles of whatever length impressed the reader as being firm and trim, without excess wordiness, so that exceptions became conspicuous,—as are soft and flabby young men among the lean and well-muscled. To assist in such conditioning, authors of longer articles are to participate in greater degree in the sharing of cost of their publication. This is not to be interpreted, however, as qualifying the basic rule that “an article should meet primarily the standard of effective presentation of the material.”

NORMAN R. STOLL,

Chairman, Editorial Committee

INDEX FOR VOLUME 28, NOS. 1-6

(For December Supplement consult its Author Index and Program.)

Acanthocephalan, new	385
Acanthor stage in acanthocephalan life histories, improved technique for study of	495
ACKERT, J. E. Natural resistance to helminthic infections	1
ACKERT, J. E. Portrait	Frontispiece
<i>Aedes aegypti</i> , a strain selected for susceptibility to <i>Plasmodium lophurae</i>	457
Alligator, strigeid trematodes of	51
America, North and South, studies on some Gordiacea of	213
American chiggers (Trombiculinae), taxonomy of some	485
AMERICAN SOCIETY OF PARASITOLOGISTS:	
17th annual meeting, Dallas, Texas, Dec. 29-31, 1941	247
Changes in membership	250
List of members	35
Preliminary announcement, 18th annual meeting, New York City, Dec. 28-30, 1942; Postponement	432; 506
Report of the Committee on Terminology of Strains of Avian Malaria	250
<i>Anopheles</i> in Georgia, macroscopic species-identification of larval	299
Antelope, American, new coccidium from	167
<i>Aporina delafondi</i> (Railliet), anoplocephalid tapeworm, the mourning dove a new host for	495
<i>Aspidogaster conchicola</i> , observations on life history and taxonomic relationships of	467
Avian Malaria, report of A.S.P. Committee on Terminology of Strains of	250
Avian plasmodia, some mosquito hosts to	127
Avian schistosomes, new species from Wisconsin and Michigan	25
Baermann apparatus in recovery of <i>Haemonchus contortus</i> larvae, efficiency of	433
Bat flea from Panama, a new species of	361
Bat infestations in human dwellings in Brazil, association of tick, <i>Ornithodoros talaje</i> with	165
BELLAMY, R. EDWARD. Observations on the macroscopic species-identification of larval <i>Anopheles</i> in Georgia	299
BELTRAN, ENRIQUE AND LUIS VARGAS. The intravenous inoculation of sporozoites of <i>Plasmodium gallinaceum</i>	246
BRACKETT, STERLING. Five new species of avian schistosomes from Wisconsin and Michigan, with the life cycle of <i>Gigantobilharzia gyrauli</i> (Brackett, 1940)	25
Brazil, association of tick, <i>Ornithodoros talaje</i> , with bat infestations in human dwellings in	165
Bugs, reduviid, reactions of man to feeding of	43
Bugs, transmission of rabbit fibroma (Shope) by haematophagous	395
BUTLER, ROBERT L., JR. AND REED O. CHRISTENSON. A simple apparatus for determining the viability of embryonated helminth ova	131
BYRD, ELON E. AND ROBERT J. REIBER. Strigeid trematodes of the alligator, with remarks on the prostate gland and terminal portions of the genital ducts	51
CABLE, R. M. AND A. V. HUNNINEN. Studies on the life history of <i>Siphodera vinalledwardsii</i> (Linton) (Trematoda: Cryptogonimidae)	407
Caribou, parasites of woodland	423
CARVALHO, JOSÉ CANDIDO M. Note on the association of the tick, <i>Ornithodoros talaje</i> (Guérin-Meneville), with bat infestations in human dwellings in Brazil	165

CARVALHO, JOSÉ CANDIDO M. Studies on some Gordiacea of North and South America	213
Cats, natural infection with <i>Centrocestus armatus</i>	285
Cattle, two parasites of, occurring in horse	245
<i>Centrocestus formosanus</i> (Nishigori, 1924), metacercaria and adult of	285
Cestode, new, from pintail duck	141
CHANDLER, ASA C. Helminths of tree squirrels in southeast Texas	135
CHANDLER, ASA C. <i>Mesocestoides manteri</i> n. sp. from a lynx, with notes on other North American species of <i>Mesocestoides</i>	227
CHANDLER, ASA C. The helminths of raccoons in east Texas	255
CHEN, H. T. The metacercaria and adult of <i>Centrocestus formosanus</i> (Nishigori, 1924), with notes on the natural infection of rats and cats with <i>C. armatus</i> (Tanabe, 1922)	285
Chicken, excystation of coccidial oöcysts of	426
Chickens, periodicity of oöcyst discharge in coccidial infection of	346
Chiggers (Trombiculinae), taxonomy of some American	485
CHRISTENSEN, N. O. (see Roth and Christensen)	245
CHRISTENSON, REED O. (see Butler and Christenson)	131
Coccidial infection of chickens, periodicity of oöcyst discharge in	346
Coccidial oöcysts of the chicken, excystation of	426
Coccidian parasite of aquatic oligochaete	424
Coccidium, <i>Eimeria antelocaprae</i> , from American antelope	167
<i>Cochlosoma rostratum</i> from the turkey	349
Cockroach, observations on <i>Plistophora kudoï</i> , a microsporidian of	399
COOK, ARTHUR H. (see Goble and Cook)	451
<i>Cooperia oncophora</i> of cattle occurring in horse	245
Copper salts, organic, tests upon <i>Strongylus vulgaris</i>	168
CORT, W. W. Robert William Hegner (1880-1942)	175
CORT, W. W. (see Olivier and Cort)	75
<i>Cotylurus communis</i> (Hughes), test of life cycle described for	75
<i>Cryptocotyle lingua</i> (Creplin, 1825), life history experiments with	91
<i>Cryptocotyle lingua</i> , 7-year-old infection in winkle	350
CULBERTSON, JAMES T. AND WALTER R. KESSLER. Age resistance of mice to <i>Trypanosoma cruzi</i>	155
CULBERTSON, JAMES T. Active immunity in mice against <i>Trichinella spiralis</i> ..	197
CULBERTSON, JAMES T. Passive transfer of immunity to <i>Trichinella spiralis</i> in the rat	203
Cultivation of <i>Trichomonas foetus</i> , a new thermostabile medium for prolonged bacteria-free	428
Culture, growth of <i>E. histolytica</i> in, effect of sulfonamide compounds on	441
Culture of <i>Neaplectana chresima</i> , a new nematode parasitic in insects	123
Culture of <i>Trichomonas vaginalis</i> , physiology of a bacteria-free	369
<i>Cyclura cornuta</i> , iguana, new nematode from	165
<i>Cysticercus fasciolaris</i> , age resistance of albino rat to	207
DEEDS, FLOYD AND JOHN O. THOMAS. Studies on phenothiazine. XI. The excretion of phenothiazone	363
Dermatitis-producing schistosomes of eastern Massachusetts	103
<i>Dero limosa</i> , oligochaete, a new coccidian parasite of	424
DINABURG, A. G. The efficiency of the Baermann apparatus in the recovery of larvae of <i>Haemonchus contortus</i>	433
Dove, mourning, new host for <i>Aporina delafondi</i>	495
Duck, pintail, new cestode from	141
<i>Echinostomum callawayensis</i> Barker, life cycle of	431
Ectoparasite resistance, vitamins in relation to	170
Editorial Committee, Chairman. Notice to members, subscribers, and authors ..	495

Eggs, nematode, buoyancy of	95
EMLEN, J. T., JR. (see Spurlock and Emlen)	341
<i>Endamoeba histolytica</i> , duration of human infection with	93
<i>Endamoeba histolytica</i> , effect of sulfonamide compounds on growth of, in culture	441
ERICKSON, ARNOLD B. AND P. R. HIGHBY. Parasites of the woodland caribou	423
EVANS, T. C. (see Levin and Evans)	477
EWING, H. E. Remarks on the taxonomy of some American chiggers (Trombiculinae), including the descriptions of new genera and species	485
Excystation of coccidial oöcysts of the chicken	426
FINLAY, C. E. Carlos Finlay and yellow fever (communication)	172
FISCHTHAL, JACOB H. A <i>Paragordius</i> larva (Gordiacea) in a trematode	167
FISCHTHAL, JACOB H. Three new species of <i>Phyllodistomum</i> (Trematoda: Gorgoderidae) from Michigan fishes	269
FISCHTHAL, JACOB H. <i>Triganodistomum hypentelii</i> n. sp. (Trematoda: Lissorchidiidae) from the hog sucker, <i>Hypentelium nigricans</i> (Le Sueur)	389
Fishes, Illinois, two new species of Myxosporidia from	83
Fishes, Michigan, new species of <i>Phyllodistomum</i> from	269
Flea, bat, from Panama, a new species of	361
Flicker, red-shafted, <i>Hypodectes chapini</i> n. sp. (Acarina) from	341
Florida Industrial School, intestinal parasites in boys of the	169
FORBES, WILLIAM C. Helminths from the Norway rat in northeastern Ohio	431
FULLER, H. S. AND Q. M. GEIMAN. South American cutaneous leishmaniasis in experimental animals	429
GEIMAN, Q. M. (see Fuller and Geiman)	429
Genitalia, male, musculature of, of <i>Haemonchus contortus</i>	351
Georgia, macroscopic species-identification of larval <i>Anopheles</i> from	299
GIRTH, H. B. (see Glaser, McCoy and Girth)	123
GLASER, R. W., E. E. MCCOY AND H. B. GIRTH. The biology and culture of <i>Neoplectana chresima</i> , a new nematode parasitic in insects	123
GOBLE, FRANS C. AND ARTHUR H. COOK. Notes on nematodes from the lungs and frontal sinuses of New York fur-bearers	451
GOBLE, FRANS C. <i>Crenosoma zederi</i> n. sp. (Nematoda: Metastrongyloidea), a new lungworm from the skunk (<i>Mephitis nigra</i>)	381
Gordiacea, a <i>Paragordius</i> larva in a trematode	167
Gordiacea of North and South America	213
GREENFIELD, SYLVIA H. Age resistance of the albino rat to <i>Cysticercus fasciolaris</i>	207
Gregarines of <i>Tenebrio molitor</i> larvae, study of temperature effects on	233
Grouse, ruffed, new parasite records from	92
Gull, black-headed (<i>Larus ridibundus</i>), immunity reaction in, infected with <i>Maritrema oöcysta</i>	423
<i>Haemonchus contortus</i> , efficiency of Baermann apparatus in recovery of larvae of	433
<i>Haemonchus contortus</i> , musculature of male genitalia of	351
HARKEMA, REINARD. <i>Pharyngostomoides procyonis</i> n.g., n. sp. (Strigeida), a trematode from the raccoon in North Carolina and Texas	117
HARKEMA, REINARD. The mourning dove, a new host of the anoplocephalid tapeworm, <i>Aporina delafondi</i> (Railliet)	495
HARTWELL, WAYNE M. Conservation of scholarly journals (communication)	174
HAUSCHKA, T. S. AND M. I. PENNYPACKER. <i>Adelina deronis</i> n. sp., a new coccidian parasite of the aquatic oligochaete, <i>Dero limosa</i>	424
Hawk, Swainson's, new spirurid nematode from	241
HAWKINS, PHILIP A. <i>Sigmodon hispidus hispidus</i> , a new host for the strobilocercus of <i>Taenia taeniaeformis</i>	94
HEGNER, ROBERT WILLIAM. (1880-1942)	175
Helminth ova, determining viability of embryonated	131

Helminthic infections, natural resistance to	1
Helminths from Norway rat in northeastern Ohio	431
Helminths of raccoons in east Texas	255
Helminths of tree squirrels in southeast Texas	135
HENDERSON, M. E. (see Threlkeld and Henderson)	351
HERBER, E. C. Life history studies on two trematodes of the subfamily Notocotylinæ	179
HERDE, KARL E. A new spiruroid nematode, <i>Thelazia buteonis</i> , from Swainson's hawk	241
HIGHBY, P. R. (see Erickson and Highby)	423
HINSHAW, W. R. (see McNeil and Hinshaw)	349
Hogs in New Orleans area, incidence of <i>T. spiralis</i> in	223
Horse, occurrence in, of two nematode species of cattle	245
Host, nutritional requirements of the fowl cestode as demonstrated by starva- tion of	319
HUIZINGA, HENRY. <i>Eimeria antelocaprae</i> , a new coccidium from the American antelope	167
HUNNINEN, A. V. (see Cable and Hunninen)	407
Hydrogen ion concentration, effect of, on population of a bacteria-free culture of <i>Trichomonas vaginalis</i>	369
<i>Hypentelium nigricans</i> (Le Sueur), hog sucker, a new trematode from	389
Identification of larval <i>Anopheles</i> in Georgia	299
Iguana, <i>Cyclura cornuta</i> , a new nematode from	165
Illinois, new species of Myxosporidia from fishes of	83
Immunity, active, in mice against <i>Trichinella spiralis</i>	197
Immunity reaction in gull infected with <i>Maritrema oocysta</i>	423
Immunity to <i>Trichinella spiralis</i> in rat, passive transfer of	203
Insects, new nematode parasitic in	123
Intestinal parasites in boys	169
Intestinal protozoa, duration of human infection with	93
Intravenous inoculation of sporozoites of <i>Plasmodium gallinaceum</i>	246
ISHII, NOBUTARO. New parasite records from the ruffed grouse	92
JOHNSON, GARTH. Physiology of a bacteria-free culture of <i>Trichomonas</i> <i>vaginalis</i> . IV. Effect of hydrogen ion concentration and oxygen tension on population	369
Journals, conservation of scholarly	174
KARTMAN, LEO. A note on vitamins in relation to ectoparasite resistance	170
KESSLER, WALTER R. (see Culbertson and Kessler)	155
KIRBY, HAROLD. A parasite of the macronucleus of <i>Vorticella</i>	311
KIRSNER, JOSEPH B. (see Rodaniche and Kirsner)	441
KOHL, GLEN M. Siphonaptera: <i>Ptilopsylla dunni</i> , a new species of bat flea from Panama	361
<i>Larus ridibundus</i> L., black-headed gull, infected with <i>Maritrema oocysta</i> , im- munity reaction in	423
Leishmaniasis, South American cutaneous, in experimental animals	429
LEVIN, A. J. AND T. C. EVANS. The use of roentgen radiation in locating an origin of host resistance to <i>Trichinella spiralis</i> infections	477
LEVINE, P. P. Excystation of coccidial oöcysts of the chicken	426
LEVINE, P. P. The periodicity of oöcyst discharge in coccidial infection of chickens	346
Life cycle of <i>Cotylurus communis</i> (Hughes)	75
Life cycle of <i>Echinostomum callawayensis</i> Barker	431
Life cycle of <i>Gigantobilharzia gyrauli</i> (Brackett, 1940)	25

Life histories, acanthocephalan, improved technique for study of acanthor stage in	495
Life history experiments with <i>Cryptocotyle lingua</i> (Creplin, 1825)	91
Life history of <i>Aspidogaster conchicola</i>	467
Life history of <i>Porocephalus crotali</i>	277
Life history of <i>Siphodera vinaldedwardsii</i> (Linton)	407
Life history studies on two trematodes (Notocotylinae)	179
<i>Littorina littorea</i> L., wrinkle, 7-year-old infection of <i>Cryptocotyle lingua</i> in	350
Lizard, <i>Tupinambis teguixin</i> , from South America, <i>Hemogregarina triatomae</i> n. sp. from	147
Louisiana muskrat, life history of <i>Porocephalus crotali</i> from	277
Louisiana muskrats, parasitological survey of	348
Lungs, nematodes from	451
Lungworm, new, from the skunk	381
Lynx, new cestode from	227
MACDOUGALL, MARY MURIEL. A study of temperature effects on gregarines of <i>Tenebrio molitor</i> larvae	
	233
<i>Macracanthorhynchus hirudinaceus</i> , chemistry of	315
MACY, RALPH W. The life cycle of the trematode <i>Echinostomum callawayensis</i> Barker	431
Malaria, a strain of <i>Aedes aegypti</i> selected for susceptibility to <i>Plasmodium</i> <i>lophurae</i>	457
Malaria, report of A.S.P. Committee on Terminology of Strains of Avian	250
Man, association of tick, <i>Ornithodoros talaje</i> , with bat infestations in dwellings of	165
Man, duration of infection with intestinal protozoa	93
Man, intestinal parasites in boys of the Florida Industrial School	169
Man, reactions to feeding of reduviid bugs	43
<i>Maritrema oocysta</i> Lebour, 1907, on immunity reaction in gull infected with	423
Massachusetts, studies on dermatitis-producing schistosomes in eastern	103
McCoy, E. E. (see Glaser, McCoy and Girth)	123
McNEIL, E. AND W. R. HINSHAW. <i>Cochlosoma rostratum</i> from the turkey	349
MEGLITSCH, PAUL A. On two new species of Myxosporidia from Illinois fishes	83
MELONEY, HENRY E. The duration of human infection with <i>Endamoeba histo-</i> <i>lytica</i> and other intestinal protozoa	93
<i>Mephitis nigra</i> , skunk, a new lungworm from	381
<i>Mesocestoides</i> , new species and notes on North American species of	227
Mice, active immunity against <i>Trichinella spiralis</i>	197
Mice, age resistance of, to <i>Trypanosoma cruzi</i>	155
Mice, device for administering particulate material to	92
Michigan fishes, new species of <i>Phyllodistomum</i> from	269
Michigan, new species of avian schistosomes from	25
Microsporidian of cockroach, observations on <i>Plistophora kudoï</i>	399
MOHAN, BADRI NATH (see Russell and Mohan)	127
MOORE, DONALD V. An improved technique for the study of the acanthor stage in certain acanthocephalan life histories	495
Mosquito hosts to avian plasmodia	127
Musculature of male genitalia of <i>Haemonchus contortus</i>	351
Muskrats, Louisiana, parasitological survey of	348
Myxosporidia, two new species from Illinois fishes	83
Nematode eggs, buoyancy of	95
Nematodes from lungs and frontal sinuses of New York fur-bearers	451
<i>Neoapectana chresima</i> , new nematode parasitic in insects; biology and culture of	123
<i>Neotoma</i> , new <i>Nematodirus</i> from	159
New genera (indicated *) and new species (volume 28, 1942):	
* <i>Acomatacarus arizonensis</i> Ewing	490
<i>Adelina deronisi</i> Hauschka and Pennypacker	424

<i>Chloromyxum opladeli</i> Meglitsch	83
<i>Chloromyxum thompsoni</i> Meglitsch	88
* <i>Comatacarus</i> Ewing	489
<i>Comatacarus americanus</i> Ewing	490
<i>Comatacarus occidentalis</i> Ewing	489
<i>Crenosoma zederi</i> Goble	381
<i>Dirofilaria tenuis</i> Chandler	262
<i>Eimeria antelocaprae</i> Huizinga	168
<i>Filicollis altmani</i> Perry	386
<i>Gigantobilharzia lawayi</i> Brackett	33
<i>Gnathostoma procyonis</i> Chandler	260
<i>Haemogregarina triatomae</i> Osimani	152
<i>Hymenolepis mastigopraedita</i> Polk	141
<i>Hypodectes chapini</i> Spurlock and Emlen	342
<i>Mesocestoides manteri</i> Chandler	229
<i>Microfilaria alpha</i> Chandler	139
<i>Microfilaria beta</i> Chandler	139
<i>Molineus barbatus</i> Chandler	259
<i>Nematodirus tortuosus</i> Tucker	159
<i>Neoapectana chresima</i> Steiner	123
* <i>Neochordodes</i> Carvalho	215
<i>Neochordodes moraisi</i> Carvalho	219
<i>Notocotylus stagnicolae</i> Herber	188
<i>Oochoristica procyonis</i> Chandler	256
<i>Paragordius esavianus</i> Carvalho	218
<i>Paralaeuris cuckleri</i> Walton	166
* <i>Pharyngostomoides procyonis</i> Harkema	117
<i>Phyllodistomum etheostomae</i> Fischthal	Supplement, Abstract 28
<i>Phyllodistomum nocomis</i> Fischthal	273
<i>Phyllodistomum notropidis</i> Fischthal	271
<i>Phyllodistomum semotili</i> Fischthal	269
<i>Pseudobilharziella burnetti</i> Brackett	29
<i>Pseudobilharziella horiconensis</i> Brackett	29
<i>Pseudobilharziella kegonensis</i> Brackett	28
<i>Pseudobilharziella waubesensis</i> Brackett	27
* <i>Pseudochordodes</i> Carvalho	216
<i>Pseudochordodes manteri</i> Carvalho	217
* <i>Pseudocrocodilicola americanense</i> Byrd and Reiber	55
<i>Pseudocrocodilicola georgiana</i> Byrd and Reiber	56
<i>Pseudoneodiplostomum acetabulata</i> Byrd and Reiber	51
<i>Ptilopsylla dunni</i> Kohls	361
<i>Raillietina</i> (<i>Raillietina</i>) <i>bakeri</i> Chandler	136
<i>Schöngastia lynni</i> Ewing	487
<i>Stempellia moniezi</i> Jones	Supplement, Abstract 5
<i>Strongyloides robustus</i> Chandler	138
<i>Synhimantus longigutturata</i> Chandler	260
<i>Thelazia buteonis</i> Herde	241
<i>Triganodistomum hypentelii</i> Fischthal	389
<i>Trombicula californica</i> Ewing	488
<i>Trombicula rohweri</i> Ewing	488
<i>Walchia americana</i> Ewing	491
New Orleans area, incidence of <i>Trichinella spiralis</i> in hogs and rats of	223
New York fur-bearers, nematodes from lungs and frontal sinuses of	451
North Carolina, new trematode from raccoon in	117
Notocotylineae, trematode subfamily, two life history studies of	179
Nutritional requirements of the fowl cestode, <i>Raillietina cesticillus</i>	319

Ohio, helminths from Norway rat in northeastern	431
Oligochaete, <i>Dero limosa</i> , a new coccidian parasite of the aquatic	424
OLIVIER, LOUIS and W. W. COURT. An experimental test of the life cycle described for <i>Cotylurus communis</i> (Hughes)	75
<i>Ornithodoros talaje</i> , tick, associated with bat infestations in human dwellings ..	165
OSIMANI, J. J. <i>Haemogregarina triatomae</i> n. sp. from a South American lizard <i>Tupinambis teguixin</i> transmitted by the reduviid <i>Triatoma rubrovaria</i>	147
<i>Ostertagia ostertagi</i> of cattle occurring in horse	245
Ova, helminth, determining viability of embryonated	131
Ova, helminth, in formalinized feces, recovery of	345
Oxygen tension, effect of, on population of a bacteria-free culture of <i>Trichomonas vaginalis</i>	369
Panama, new species of bat flea from	361
<i>Paragordius</i> larva in a trematode	167
Parasites, intestinal, in boys	169
Parasites of the woodland caribou	423
PENN, GEORGE H., JR. Parasitological survey of Louisiana muskrats	348
PENN, GEORGE H., JR. The life history of <i>Porocephalus crotali</i> , a parasite of the Louisiana muskrat	277
PENNER, LAWRENCE R. Studies on dermatitis-producing schistosomes in eastern Massachusetts, with emphasis on the status of <i>Schistosomatum pathlocopticum</i> Tanabe, 1923	103
PENNYPACKER, M. I. (see Hauschka and Pennypacker)	424
PERES, CHARLES E. <i>Trichinella spiralis</i> . II. Incidence of infection in hogs and rats in the New Orleans area	223
Periodicity of oöcyst discharge in coccidial infection of chickens	346
PERRY, MARY LOUISE. A new species of the acanthocephalan genus <i>Filicollis</i> ..	385
Phenothiazine, studies on: excretion of phenothiazone	363
PHILIP, CORNELIUS B. Mechanical transmission of rabbit fibroma (Shope) by certain haematophagous bugs	395
<i>Plasmodium cathemerium</i> , oral transmission of, by means of tissues	245
<i>Plasmodium gallinaceum</i> , intravenous inoculation of sporozoites of	246
<i>Plasmodium gallinaceum</i> , mosquito hosts of	127
<i>Plasmodium lophurae</i> , avian malaria parasite, strain of <i>Aedes aegypti</i> selected for susceptibility to	457
<i>Plistophora kudoï</i> , microsporidian of cockroach, observations on	399
POLK, SEIGUL J. <i>Hymenolepis mastigopraedita</i> , a new cestode from a pintail duck	141
<i>Porocephalus crotali</i> , from Louisiana muskrat, life history of	277
Protozoa, intestinal, duration of human infection with	93
Raccoon in North Carolina and Texas, new trematode from	117
Raccoons in east Texas, helminths of	255
<i>Raillietina cesticillus</i> (Molin), nutritional requirements of	319
RAMSEY, JUANITA (see Sprague and Ramsey)	399
Rat, albino, age resistance of, to <i>Cysticercus fasciolaris</i>	207
Rat, <i>Neotoma</i> , new <i>Nematodirus</i> from	159
Rat, Norway, helminths from, in northeastern Ohio	431
Rat, passive transfer of immunity to <i>Trichinella spiralis</i> in the	203
Rats, natural infection with <i>Centrocestus armatus</i>	285
Rats of New Orleans area, incidence of <i>T. spiralis</i> in	223
Reduviid bugs, reactions of man to feeding of	43
Reduviid, <i>Triatoma rubrovaria</i> , transmitter of hemogregarine of lizard	147
REIBER, ROBERT J. (see Byrd and Reiber)	51
REID, W. M. Certain nutritional requirements of the fowl cestode <i>Raillietina cesticillus</i> (Molin) as demonstrated by short periods of starvation of the host ..	319

Resistance, age, of albino rat to <i>Cysticercus fasciolaris</i>	207
Resistance, age, of mice to <i>Trypanosoma cruzi</i>	155
Resistance, host, to <i>Trichinella spiralis</i> , use of roentgen radiation in locating origin of	477
Resistance to helminthic infections, natural	1
Resistance, vitamins in relation to ectoparasite	170
RODANICHE, ENID C. AND JOSEPH B. KIRSNER. The effect of sulfonamide compounds on the growth of <i>Endamoeba histolytica</i> in culture	441
Roentgen radiation, use in locating origin of host resistance to <i>Trichinella spiralis</i>	477
ROTH, HANS AND N. O. CHRISTENSEN. Occurrence in the horse of two parasites of cattle, <i>Ostertagia ostertagi</i> (Stiles, 1892) and <i>Cooperia oncophora</i> (Railliet, 1898)	245
ROTHSCHILD, MIRIAM. A further note on life history experiments with <i>Cryptocotyle lingua</i> (Creplin, 1825)	91
ROTHSCHILD, MIRIAM. A note on immunity reaction in the black-headed gull (<i>Larus ridibundus</i> L.) infected with <i>Maritrema oocysta</i> Lebour, 1907	423
ROTHSCHILD, MIRIAM. A seven-year-old infection of <i>Cryptocotyle lingua</i> Creplin in the wrinkle <i>Littorina littorea</i> L.	350
RUSSELL, PAUL F. AND BADRI NATH MOHAN. Some mosquito hosts to avian plasmodia with special reference to <i>Plasmodium gallinaceum</i>	127
SAURWEIN, JEAN (see von Brand and Saurwein)	315
SAWITZ, WILLI. The buoyancy of certain nematode eggs	95
<i>Schistosomatum pathlocopticum</i> Tanabe, 1923, studies on	103
Schistosomes, avian, from Wisconsin and Michigan	25
Schistosomes, dermatitis-producing, studies on	103
SCHNEIDER, MORRIS D. A new thermostable medium for the prolonged bacteria-free cultivation of <i>Trichomonas foetus</i>	428
<i>Sigmodon h. hispidus</i> , new host for strobilocercus of <i>Taenia taeniaeformis</i>	94
Sinuses, frontal, nematodes from	451
<i>Siphodera vinaledwardsii</i> , studies on life history of	407
Skunk, <i>Mephitis nigra</i> , a new lungworm from	381
South America, <i>Hemogregarina triatomae</i> n. sp. from lizard, <i>Tupinambis teguixin</i> , of	147
South American cutaneous leishmaniasis in experimental animals	429
SPRAGUE, VICTOR AND JUANITA RAMSEY. Further observations on <i>Plistophora kudoï</i> , a microsporidian of the cockroach	399
SPURLOCK, G. M. AND J. T. EMLEN, JR. <i>Hypodectes chapini</i> n. sp. (Acarina) from the red-shafted flicker	341
Squirrels, tree, helminths of, in southeast Texas	135
Strains of Avian Malaria, report of A.S.P. Committee on Terminology of	250
<i>Strongylus vulgaris</i> , tests with organic copper salts upon	168
Sucker, hog, <i>Hypentelium nigricans</i> , a new trematode, <i>Triganodistomum hypentelii</i> , from	389
Sulfonamide compounds, effect on growth of <i>E. histolytica</i> in culture	441
SUMMERS, WILLIAM A. A modification of zinc sulfate centrifugal flotation method for recovery of helminth ova in formalinized feces	345
SUMMERS, WILLIAM A. Intestinal parasites in boys of the Florida Industrial School	169
Survey, parasitological, of Louisiana muskrats	348
Susceptibility to <i>Plasmodium lophurae</i> , a strain of <i>Aedes aegypti</i> selected for	457
<i>Taenia taeniaeformis</i> , strobilocercus of, <i>Sigmodon h. hispidus</i> a new host for	94
Temperature effects on gregarines of <i>Tenebrio molitor</i> larvae	233
Terminology of Strains of Avian Malaria, report of A.S.P. Committee on	250
Texas, east, helminths of raccoons in	255
Texas, new trematode of raccoon in	117

Texas, southeast, helminths of tree squirrels in	135
THOMAS, JOHN O. (see DeEds and Thomas)	363
THRELKELD, W. L. AND M. E. HENDERSON. Notes on the musculature of the male genitalia of <i>Haemonchus contortus</i>	351
Tick, <i>Ornithodoros talaje</i> , associated with bat infestations in human dwellings	165
TRAGER, WILLIAM. A strain of the mosquito <i>Aedes aegypti</i> selected for susceptibility to the avian parasite <i>Plasmodium lophurae</i>	457
Transmission of rabbit fibroma (Shope) by certain hematophagous bugs	395
Transmission, oral, of <i>Plasmodium cathemerium</i> by means of tissues	245
Trematode, new, from raccoon in North Carolina and Texas	117
Trematode, <i>Paragordius</i> larva in a	167
Trematodes, life history studies on two Notocotylineae	179
Trematodes, strigeid, of the alligator	51
<i>Triatoma rubrovaria</i> , reduviid transmitting hemogregarine of lizard	147
<i>Trichinella spiralis</i> , active immunity in mice against	197
<i>Trichinella spiralis</i> , incidence in hogs and rats in New Orleans area	223
<i>Trichinella spiralis</i> in the rat, passive transfer of immunity to	203
<i>Trichinella spiralis</i> , use of roentgen radiation in locating origin of host resistance to	477
<i>Trichomonas foetus</i> , a new medium for prolonged bacteria-free cultivation of	428
Trombiculinae, American chiggers, taxonomy of some	485
<i>Trypanosoma cruzi</i> , age resistance of mice to	155
TUCKER, HAL. <i>Nematodirus tortuosus</i> n. sp. (Nematoda) from the rat, <i>Neotoma</i>	159
<i>Tupinambis teguixin</i> , South American lizard, <i>Hemogregarina triatomae</i> n. sp. from, transmitted by <i>Triatoma rubrovaria</i>	147
Turkey, <i>Cochlosoma rostratum</i> from the	349
VARGAS, LUIS (see Beltran and Vargas)	246
Viability of embryonated helminth ova	131
Vitamins in relation to ectoparasite resistance	170
VON BRAND, THEODOR AND JEAN SAURWEIN. Further studies upon the chemistry of <i>Macracanthorhynchus hirudinaceus</i>	315
<i>Vorticella</i> , a parasite of macronucleus of	311
WALLACE, F. G. A device for administering particulate material to mice	92
WALTON, A. C. <i>Paralaeuris cuckleri</i> n. sp. (Nematoda) from the iguana (<i>Cyclura cornuta</i>)	165
WHITLOCK, J. H. Studies upon <i>Strongylus vulgaris</i> . VI. Tests with organic copper salts	168
WILLIAMS, CHARLES O. Observations on the life history and taxonomic relationships of the trematode <i>Aspidogaster conchicola</i>	467
Winkle, <i>Littorina littorea</i> , 7-year-old infection of <i>Cryptocotyle lingua</i> in	350
Wisconsin, new species of avian schistosomes from	25
WOLFSON, FRUMA. Oral transmission of <i>Plasmodium cathemerium</i> by means of tissues	245
WOOD, SHERWIN F. Reactions of man to the feeding of reduviid bugs	43
Yellow fever, Carlos Finlay and	172
Zinc sulfate centrifugal flotation modification for recovery of helminth ova in formolinized feces	345

AMERICAN SOCIETY OF PARASITOLOGISTS

Announcement of Postponement of the New York Meeting

On November 28, a letter was received from Dr. F. R. Moulton, Permanent Secretary, stating that the Executive Committee of the American Association for the Advancement of Science had voted to postpone the New York meeting, "in compliance with the request of the Office of Defense Transportation. . . . As yet no plans have been formulated for holding the meeting in the immediate future."

By vote of Council of the American Society of Parasitologists, the 18th Annual Meeting of the Society, scheduled to be held with the New York meeting of the A. A. A. S., was in turn postponed.

JAMES T. CULBERTSON,
Secretary

December 1, 1942.